VU Research Portal

Chlorinated micropollutants in products

van Hattum, A.G.M.; Swart, C.P.; Lamoree, M.H.; ven der Linden, S.; Senhorst, H.; de Voogt, P.; Tukker, A.

2004

Institut voor Milieuvraagstukken / Institute for Environmental Studies

IVM

Chlorinated Micropollutants in Products

B. van Hattum
K. Swart
M. Lamoree
S. van der Linden
H. Senhorst
P. de Voogt
A. Tukker

vrije Universiteit amsterdam

Chlorinated Micropollutants in Products

B. van Hattum [1], K. Swart [1], M. Lamoree [1], S. van der Linden [2], H. Senhorst [3], P. de Voogt [4] and A. Tukker [5]

[1] IVM, Vrije Universiteit, Amsterdam

[2] BDS, Amsterdam

[3] RIZA, Rijkswaterstaat, Lelystad

[4] IBED, University of Amsterdam

[5] TNO-STB, Delft

Chlorine Chain Follow-up Research Programme on chlorinated organic microcontaminants (OVOC)

E-04/05

September, 2004

Commissioned by the Ministry of Housing, Spatial Planning, and the Environment (VROM, DGM; contract nr. 99230300), the Ministry of Transport, Public Works, and Water Management (V&W, Directorate Water), the Association of the Dutch Chemical Industry (VNCI) and the Netherlands Society for Nature and Environment (SNM).

This report was approved by the OVOC steering committee.

IVM

Institute for Environmental Studies
Vrije Universiteit
De Boelelaan 1087
1081 HV Amsterdam
The Netherlands

Tel. ++31-20-4449 555
Fax. ++31-20-4449 553
E-mail: info@ivm.falw.vu.nl

OVOC Reports Phase-2:

Chlorinated Micropollutants in Aquatic Effluents

- Part-1 Screening studies (WP5&6)
- Part-2 Biodegradation studies (WP8)
- Part-3 TIE studies (WP7&8)
- Part-4 In-plant TIE Studies (WP9)

Chlorinated Micropollutants in Atmospheric Emissions

Chlorinated Micropollutants in Products

Contents

Preface

In this report the results are presented of the chemical and biological screening of a selection of technical and commercial products in the framework of the OVOC-study on chlorinated organic micropollutants, commissioned by the Ministry of Housing, Spatial Planning, and the Environment (VROM, DGM; contract nr. 99230300), the Ministry of Transport, Public Works, and Water Management (V&W, Directorate Water), the Association of the Dutch Chemical Industry (VNCI), and the Netherlands Society for Nature and Environment (SNM).

Draft versions of this report, presented and discussed at a technical workshop held 14-15 May, 2003 in Den Haag, have been discussed with suppliers of the products, members of the BOVOC steering committee, and invited experts in the period between May 2003-July 2004. In several cases comparisons could be made with relevant information from internal studies provided by the suppliers. This information was included in the report for some products. For readers not familiar with concentration units a conversion table is included in the Annex section (Appendix-V), which also explains the different units used for expression of dioxin concentrations and response of the DR-CALUX® bioassay.

The authors gratefully acknowledge the willingness and kind co-operation of the companies, who participated in the study. We further acknowledge the contributions to this study by laboratories subcontracted, helpful colleagues and the comments made by members of steering committee and invited experts.

Summary

In this report the results are presented of the chemical and biological screening of 11 commercial products in the framework of work packages 5 and 6 (Phase-2) of the OVOC-project on potential emissions of chlorinated organic micropollutants (PBTs: persistent, bioaccumulative, toxic compounds) from the chlorine chain. In a previous priority setting study (Phase-1) eleven potentially relevant products were selected. Nine products resulted from a systematic prioritization based on data available from national regulatory agencies and previous studies. Two products (chloroprene rubber, triclosan) were selected as 'wildcards' based on proposals from national environmental NGOs.

In order to reveal and identify possible chlorinated microcontaminants in the selected products, extracts, solutions or vaporization residues of these products were subjected to biological screening for total dioxin-like response with the DR-CALUX® bioassay, and to chemical screening with GC-MS, followed by automated comparison, using AMDIS software, with a large database of mass spectra (NIST).

A DR-CALUX® response (after acidic clean-up) was observed at well quantifiable levels in chloroprene rubber extracts (1700 pg bio-TEQ/g)[1] and to a lesser extent in hydrochloric acid and trichloroethylene (2-10 pg bio-TEQ/g). In two other products (EDC, PER) a significant response was detected at levels close to the limit of detection between 0.01-0.07 pg bio-TEQ/g. Although no reliable usage figures are available for chloroprene rubber, a tentative estimation of the annual loads or fluxes of dioxin equivalents involved indicated possible contributions in the order 1-2 g bio-TEQ/yr for chloroprene rubber, approximately 0.01-0.03 g bio-TEQ/yr for HCl, taking into account that this sample can not be extrapolated to the Dutch HCl market, <0.005 g bio-TEQ/yr for TRI, <0.002 g bio-TEQ/yr for EDC, and less than 0.0001 g bio-TEQ/yr for PER. Compared to the total national emissions of dioxins (I-TEQs) in the Netherlands in 1999 of 38 g I-TEQ/yr the possible contribution from products seems only significant for chloroprene rubber and of minor importance for the other responsive products.

Additional chemical analysis with HRGCMS confirmed the presence of polychlorinated dibenzodioxins (PCDDs) and dibenzofurans (PCDFs) in the three most responsive products at concentrations of 90 pg WHO-TEQ/g for chloroprene rubber, 0.3 pg WHO-TEQ/g for HCl and 0.7 pg WHO-TEQ/g for trichloroethylene. Only a limited fraction of the bioassay-based response therefore could be attributed to PCDD/Fs, ranging from 5% for chloroprene rubber to 17-21 % (HCl). In the case of TRI the presence of a tetrachlorinated naphthalene compound was confirmed with HRGCMS, which may imply possible contributions from responsive polychlorinated naphthalene congeners. However, the major part of the DR-CALUX® response could not be attributed in this study to identified chlorinated compounds in the GC-MS screening or to identified non-chlorinated

---

[1] Conventions for expressing dioxin equivalent concentrations determined with biological or chemical methods are explained in Annex-5.

compounds with known response in the DR-CALUX® assay, such as e.g. some PAHs in the case of chloroprene rubber.

Similar as in the aquatic and atmospheric studies, the GC-MS screening studies demonstrated the presence of complex mixtures of impurities. In 8 of the 11 products 3-17 chlorinated compounds (per product) could be tentatively identified[2] with an acceptable reliability at indicative concentration levels of 0.1-100 mg/kg for chloroprene rubber (Soxhlet-extractable compounds) and 10^{-5}-1 mg/kg for the seven other products.

Further manual examination of non-assigned components in the AMDIS-NIST analysis revealed the presence of multiple chlorinated compounds, especially in chloroprene rubber, allylchloride, TRI, and MCA. In many cases homologous series of related compounds with a varying degree of chlorination were found, which except for MCA could partly be attributed to the specific production process. Preliminary estimates of total annual loads of these impurities (including the tentatively identified compounds) in the products varied from <0.1-1.5 kg for TRI and HCl, 0.2-2 kg for allylchloride, 0.8-8 for EDC, 1-11 kg for MCA, and 20-200 kg for chloroprene rubber. For the other compounds (PER and DCM) annual loads were estimated to be <0.04 kg and <0.02 kg respectively.

Specific compound classes to be addressed in the final phase of the OVOC project include: chlorinated phenols (in TRI and HCl), chlorinated (alkylsubstituted) benzenes (in 1,2-dichloroethane, dichloromethane, HCl, MCA and chloroprene rubber), and chlorinated alkanes or alkenes (>C4 in PER, TRI and chloroprene rubber).

Due to problems related to chromatographic separation, selectivity or sensitivity the screening approach was not successful to identify chlorinated components at levels below 1% for 1,4-dichlorobenzene, triclosan, and chloroparaffins.

The fact that the most responsive product (chloroprene rubber) was one of the two products selected as 'wildcard' indicates that the priority setting procedure in the OVOC project was not comprehensive. Chlorinated impurities in imported products may need further attention

---

[2] Explanation of status of identification: p.5 and p.13.

1. Introduction

As a result of the Dutch national debate on chlorine during the 1990s and based on the results of the chlorine mass balance study covering 99% of the major fluxes of chlorine and chlorinated products (Tukker et al., 1997), initiatives were taken to start a study programme on the occurrence of unknown emissions of chlorinated micropollutants in industrial effluents and other selected emission sources. The programme was designed in the framework of the BITAC in close co-operation between the government, industry, environmental NGOs and a number of research institutes (Van Hattum et al., 1998). The main objective of this programme is to determine if unknown chlorinated PBT micropollutants (PBT, i.e. persistent, bioaccumulative, toxic compounds) are emitted from the chlorine chain, and if so to what extent.

The OVOC or chlorinated micropollutant project is a 4-year programme and consists of three phases. During the preparatory Phase-1 various background literature studies were executed (Klein et al., 2001a, 2001b), potential emission sources (aquatic effluents, atmospheric emissions and products) were reviewed and prioritised (Tukker et al., 2000a, 2000b), and chemical and biological screening methods were optimised in a pilot-study (Belfroid et al., 2000). During Phase-2 further background studies and the main experimental screening studies are being executed, focussing on industrial and other aquatic effluents (Lamoree et al., 2002, 2003; Senhorst et al., 2003a,b), atmospheric emissions (Van Hattum et al., 2003) and products that are marketed on an industrial scale (this report). Full details of the objectives, organisation and experimental approach of Phase-2 are described in the workplan (Van Hattum et al., 2000). The integration of the results and final reporting will be executed during Phase-3.

Based on updated figures from the Dutch chlorine chain study (Tukker et al., 1997), information on impurities in products from commercial suppliers, and other literature sources, a tentative estimate was made of the potential contribution of impurities in commercial products and technical mixtures. Although such impurities may be present in low concentrations (sub ppm to sub % range) in the products, this could constitute a potential significant source for chlorinated microcontaminants, especially for high volume products. For products with a usage of 1000 tonnes per year (tpa), this could imply fluxes ranging from less than a kg/year up to the tpa level. Against this background a selection of products was proposed for screening on the presence of chlorinated compounds (Tukker et al., 2000a). After consultation of a technical workshop held in May 2000 this primary selection was completed with two additional products (chloroprene rubber, triclosan) selected as ‘wildcards’ by national environmental NGOs and adopted by the BOVOC steering committee (see Table 1.1). One of the originally proposed products (chlorophenols) was removed from the selection, because major technical applications have ceased in the Netherlands.

In Table-1.1 an indication is given of the annual amounts of the products marketed in the Netherlands. In addition to data for the period before 1995 and derived from the chlorine chain study (Tukker et al. 2000b), more recent data are presented in the table for the period 2000-2003, based on figures and estimates provided by the suppliers. A distinction is made between captive and non-captive usage of products. Non-captive use of products

includes applications in end products sold on the market, such as the solvents (TRI, PER, DCM), chloroprene rubber (CR), chloroalkanes, triclosan and 1,4-dichlorobenzene. These non-captive applications were considered as the most relevant for the estimation of potential emissions during usage in disperse applications or during the stage of waste handling. Captive usage includes application in large volumes as feedstock in consecutive process steps, either at the same location (on-site captive use) or at a different location (off-site captive use) by the same, or a different company. For EDC and AC the majority of the amounts produced are used in on-site follow-up processes, i.e. the production of VCM/PVC and the production of ECH/epoxy resins respectively. The OVOC effluent and atmospheric emission studies (Lamoree et al., 2003; Van Hattum, 2004a) included a check of emissions from these processes themselves, and from literature data of the end products (Tukker et al., 2000a) it is known that impurities are hardly relevant. In order to show only the 'real' uncertainty in the fate of such potential impurities in products, for EDC and AC only the externally sold or processed amounts were taken into account in the estimation of potential emissions of impurities.

Table 1.1 Selected products and annual volumes marketed or used in the Netherlands (excluding on-site captive use[a] as intermediate or feedstock in major production processes).

Product	Usage [b] <1995 tpa	Updated figures [c] 2000-2003 tpa
Dichloromethane (DCM)	8500	4050
1,2-dichloroethane (EDC)		<80000 [d]
Perchloroethylene (PER)	< 2600	1500
Trichloroethylene (TRI)	926	<500
Hydrochloric acid (HCl)	20000 [e]	<15000 [f]
Allylchloride (AC)	< 3000 [g]	200
Monochloroacetic acid (MCA)	10000	<10000 [h]
1,4-dichlorobenzene	100 [i]	50
Chloroalkanes C_{14-17}	1400 [j]	
Triclosan		3-16 [k]
Chloroprene rubber (CR)		1000 [l]

[a] Explained in text;
[b] Estimated usage as given in Tukker et al. (2000b) based on the chlorine chain study (Tukker et al., 1997);
[c] Data for period 2000-2003 as provided by suppliers and Eurochlor;
[d] Total Dutch production > 600 ktpa, maximum non captive use high purity EDC according to permit 80 ktpa;
[e] As separated from by-products, excluding 14.6 ktpa product with high purity;
[f] Total amount as 100% product from the two plants selected for OVOC study; amount will decrease due to closure of one of the plants in 2004;
[g] Excluding captive use for ECH production and 18 ktpa exported;
[h] Estimate for total Dutch usage , including off-site captive use by the same company; amounts as 100% product;
[i] Only dichlorobenzenes, total chlorinated benzenes: 9 ktpa;
[j]: Use <1990 was estimated as 2 ktpa; no recent estimate available;
[k] Based on average consumption of 0.2-1.0 g/person/yr for northern / southern European countries;
[l] Uncertain estimate, not confirmed by supplier.

For MCA the usage figure in Table 1.1 includes a large amount of product that is applied in off-site captive use (CMC production) by the same manufacturer. HCl is a by-product of different processes. The impurities are expected to be dependent on the process. Hence, not the full amount of HCl on the Dutch market is indicated in Table 1.1, but only the amount from the processes covered in the OVOC study. The data for DCM, TRI and PER were provided by ECSA (European Chlorinated Solvent Association, Brussels, Belgium) and Eurochlor (Brussels, Belgium). For chloroprene rubber and chloroalkanes no actual usage figures for the Netherlands were available and a preliminary surrogate value of 1000 tonnes per annum (tpa) was used for chlorprene rubber to estimate the order of magnitude. For most of the products indicated in Table 1.1 the underlying assumptions of the non-captive use estimates indicated in footnotes.

The goals for the present product screening study were to identify what type of chlorinated compounds were present as impurities in the selected products and to what extent these compounds could be assigned as PBT compounds. Although in the original workplan (Van Hattum et al., 2000) the focus of the product study was restricted to chemical aspects (fractionation and identification), it was later decided to include toxicity screening with the in-vitro DR-CALUX® bioassay and to use a combined chemical and biological screening approach, consistent with the aquatic and atmospheric screening studies. Extracts or solutions of the products in hexane were:

1. Analysed with GC-MS in combination with application of the AMDIS identification software;
2. Submitted to the DR-CALUX® bioassay for the screening of total response of compounds with a dioxin-like mode of action. The three most responsive products were subjected to additional analysis of polychlorinated dibenzodioxins and furans (PCDD/Fs).

2. Methods

With the help of the VNCI (Dr. J. Bouwma) a selection of technical grades and supplier's representative for the Dutch market was made and contact persons for the selected companies were identified and invited to participate in the project. Following up on this, the contact persons were approached by IVM for confirmation of the willingness to participate and for specific technical questions regarding the feasibility to obtain samples. In agreement with all participants, information on the origins of the products was treated as confidential during the course of the project. The samples were collected in the period between May 2001 and October 2002.

2.1 Sample preparation

From solvents and volatile liquid products (dichloromethane, 1,2-dichloroethane, PER, TRI, allylchloride) an amount of 0.7-2 L was taken and concentrated to extracts of less volatile impurities using a Rotavapor installation, water bath, or gentle N_2 stream (see Table 2.1) and finally taken over or transferred to 1 ml of hexane in calibrated vials.

The water-soluble liquid products (HCl 2100 ml, MCA 900 ml) were liquid-liquid extracted with hexane, concentrated and finally transferred to 1 ml of hexane.

Solid products soluble in hexane were dissolved in hexane and brought to w/v concentrations of 10 mg/L (1,4-dichlorobenzene, triclosan) or 1000 mg/L (chloroalkanes $C_{14\text{-}17}$). For chloroprene rubber a sub sample of 10 g of finely cut material was taken and Soxhlet extracted with 200 ml hexane/acetone (3/1) for 12 hours and concentrated with a Kuderna Danish evaporation unit to 5 ml. Further concentration was not applied because of formation of precipitate, probably consisting of polymerisation products.

2.2 GC-MS and identification

GC-MS analysis was performed at IVM with similar instrumentation (HP6890 and HP5973N MSD spectrometer) and settings as applied in other workpackages (Senhorst et al., 2004; Lamoree et al., 2003). For most products the following GC parameters were used. A SGE BPX5 column with a length of 24.4 m, a diameter of 220 mm and a film thickness of 0.30 mm was applied. The injection volume was 5 µl in solvent vent mode at 40 °C. The initial column temperature was 60 °C for 3 minutes, increasing to 200 °C at a rate of 30 °C/minute. The column was kept at 200 °C for 15 minutes, followed by an increase of the temperature with 5 °C/minutes to 265 °C for 5 minutes, and then at a rate of 3 °C/minutes to a final temperature of 275 °C for 15 minutes. The total runtime was 59 minutes. For Triclosan the same column was used. The injection volume was 3 µl in hot splitless mode at 275 °C. The initial column temperature was 60 °C for 3 minutes, increasing to 280 °C at a rate of 15 °C/minutes. The total runtime was 23.67 minutes. For the chlorinated paraffins also the same column was applied. The injection volume was 3 µl in hot splitless mode at 275 °C. The initial column temperature was 40 °C for 3 minutes, increasing to 150 °C at a rate of 25 °C/minutes.

Table 2.1 Overview of preparation of samples.

Product	Amount used	Final volume in hexane	Tempe-rature	Sample preparation
Dichloromethane (DCM)	1956 ml	1 ml	40-45 °C	Rotavap to 3 ml; at 30 oC to 0.3 ml; uptake in 3 ml methanol; evap to 100 µL; uptake in 5 ml hexane evap to 1 ml
1,2-dichloroethane (EDC)	1942 ml	1 ml	40-45 °C	Rotavap to 3 ml; evap. with N_2 (30 °C) to 0,1 ml; redissolve in 5 ml hexane; evap. with N_2 (30 °C) to 1 ml
Perchlorethylene (PER)	755 ml	1 ml	75 °C	Waterbath to 3 ml; rotavap to 0.1 ml redisssolve in 5 ml hexane; evap to 1 ml
Trichloroethylene (TRI)	704 ml	1 ml	45 °C	Waterbath to 3 ml calibrated vial to 0.1 ml redissolve in hexane 5 ml evaporate to 1 ml
Hydrochloric acid (HCl)	2100 ml	1 ml	40 °C	Shakeflask with 4x200 ml hexane rotavap to 3 ml calibrated vial under N_2 to 1 ml
Allylchloride	1713 ml	1 ml	50 °C 55 °C	Waterbath to 7 ml rotavap to 0.1 ml hexane 5 ml to 1
Monochloro acetic acid (MCA)	900 ml	1 ml	40 °C	Hexane 4 x 100 ml; rotavap to 8 ml under N_2 to almost dry, uptake in 5 ml/2ml water/hexane; hexane evaporated to 1 ml
1,4-dichlorobenzene	20 mg	1 ml		In 20 ml hexane; dilution 1:100 to 10 mg/L
Chloroalkanes C_{14-17}	20 mg			In 20 ml hexane; 1 g/L
Chloroprene rubber	10 g	5 ml		Soxhlet with 200 ml hexane/acetone (3/1) for 12 hours; evap. with Kuderna Danish to 5 ml
Triclosan	20 mg	1 ml		In 20 ml hexane; dilution 1:100 to 10 mg/L

The column was kept at 150 °C for 1 minute, and after this the temperature was increased with 7 °C/minutes to 215 °C for 1 minute, and then at a rate of 5 °C/minutes to a final temperature of 280 °C for 13 minutes. The total runtime was 42.69 minutes. As carrier gas, helium was used at a constant flow rate of 1 mL/minutes. For mass spectrometric detection, data were acquired in full scan mode from m/z 50 to m/z 650, at 2 scans/s. A solvent delay for data acquisition of 2.1 minutes was used to avoid disturbances in MS detection. In each series of samples a methanol blank was measured and an alkane mixture (n-C8-C24 (NIST) in order to provide for an indication of the retention index vs. retention time, as well as a typical response factor for the apparatus.

Comparison with NIST database of spectra

For the interpretation of the complex chromatograms and mass spectra the AMDIS software package in combination with the NIST database (1998) was used (Mallard and Reed, 1997; Dugan, 2000). This method was previously employed in the OVOC study in other work packages (WP 9 and WP 7; Senhorst et al., 2003; Lamoree et al., 2003). Using this software, mass spectra are extracted from the raw GC-MS datafiles and subjected to a specific background correction technique (deconvolution) and automatically matched with the NIST mass spectral database. Based on pattern-matching statistics probable identifications are proposed and reported to an ASCI based output file. Within the list of probable identifications a manual selection was made of chlorinated compounds. As a last step, a manual comparison of extracted spectra and library spectra was applied for confirmation. The deconvolution technique is very powerful and allows identification of compounds at the ng/µl level even under conditions of poor chromatographic separation from interfering components present at order of magnitude higher concentrations (Dugan, 2000).

It should be emphasized that with this approach only those compounds that are included in the NIST database will be identified. However, as this database contains over a hundred thousand mass spectra, this is at present the most effective way of dealing with the enormous data sets.

For the components identified, concentration ranges were estimated using the peak area determined with the HP-Chemstation software, and an average response factor obtained from a standard mixture of 7 PCBs and 22 chlorinated pesticides. The coefficient of variation (CV) of the relative response factor for the 29 compounds was 47%. Due to the large uncertainties in the (unknown) response factors of the identified components estimated concentration ranges (order of magnitude) were reported as intervals of powers of 10 (e.g. 10-100 mg/kg). All concentrations for HCl and MCA are reported on a 100% weight basis, although these products are sold as diluted aqueous solutions (30% and 80% respectively).

Examination of unresolved mass spectra

As our experience in related studies (Senhorst et al., 2004) has shown that in the AMDIS/NIST analysis a large number of deconvoluted spectra cannot be matched with an existing mass spectrum in the NIST database, an additional approach was followed to examine the presence of chlorine containing fragments among the unresolved deconvoluted spectra. The approach is based on the analysis of the typical istotope (m/z) patterns of chlorine containing mass fragments, with m/z= x, x+2, x+4, …x+n for monochloro- or higher chloro-subsituted fragments (see e.g. Figure 3.1, where the cluster around m/z 266 is typical for a pentachloro fragment). The respective single ion current (SIM) signals of representative mass lines are used to find other peaks in the chomatogram and mass spectra with structural similarity. This approach was also followed in WP9 (Senhorst et al., 2004) to examine the presence of related chlorinated compounds that were overlooked by the AMDIS/NIST procedure.

The analysis of structurally related peaks of chlorinated compounds starts with the inspection of the mass spectra of one or more of the identified chlorinated compounds.

From these spectra selected two or more representative mass lines of chlorinated fragments are used, recognisable by the specific chlorine isotope pattern. By inspection of the ion chromatograms of these mass lines, other components deconvoluted by AMDIS, in which these mass lines also appear, can be found. The full mass spectrum of these components may show additional chlorine isotope clusters. These structures are in turn used to find additional chlorine containing components. By working this way a group of related chlorine containing components is found that shares one or more chlorine fragments in their mass spectrum. The groups and number of compounds found by this procedure are not comprehensive, and no full identification is possible, but it may provide an indication of substructures present and the number of chlorine atoms involved. Another approach used for trichloroethylene, where a large number of unresolved components appeared to contain chlorine clusters, was based on the formulation of possible reactions of the main building blocks. Total concentrations of these unidentified chlorinated impurities were determined using the total area of the reconstructed TIC chromatogram of these components, as calculated by the AMDIS programme, and multiplied with an average response factor derived from the mixture of 7 PCB congeners and 22 chlorinated pesticides. Due to the large uncertainties in response factors, the concentrations were reported as estimated order of magnitude intervals (powers of 10).

2.3 DR-CALUX®

The DR-CALUX® bioassay analysis was performed by BioDetection Systems (BDS). The Dioxin Responsive – Chemically Activated LUciferase eXpression (DR-CALUX®) assay comprises a genetically modified H4IIE rat hepatoma cell-line, incorporating the firefly luciferase gene coupled to dioxin responsive elements (DREs) as a reporter gene for the presence of dioxins and dioxin-like compounds (including polychlorinated dibenzodioxins (PCDDs) and materials which are widely accepted to act in a toxicologically similar manner to PCDDs such as the polychlorinated dibenzofurans (PCDFs) and the co-planar dioxin-like polychlorinated biphenyls (PCBs)). Cells that are exposed to dioxins or dioxin-like chemicals not only express proteins and enzymes that are under normal circumstances associated to the DRE, but also luciferase. By addition of the appropriate substrate for luciferase, light is emitted. The amount of light produced is proportional to the amount of ligand-AhR binding, which is related to 2,3,7,8-TCDD toxic equivalents (TEQs). In this way, the DR-CALUX® assay reports toxic equivalents (TEQs) benchmarked against 2,3,7,8-TCDD as “bio-TEQs”.

BDS received approximately 0.05 ml of the sample extracts. Of the received extracts, each sample was split into two sub samples volumetrically: half of the sub samples were transferred to 25 µl of DMSO, while the other half was cleaned over a multi-layered glass column. These columns (i.d. 10 mm) consisted of (from top to bottom): 0.5 g anhydrous sodium sulphate, 5 g 20% H2SO4 and 5 g 33% H_2SO_4 deactivated silica. The columns were vibrated to pack them and then rinsed with 20 ml of hexane: diethylether (97:3, v/v) and eluted with 40 ml of the same solvent mix. The cleaned extract was evaporated under a gentle N2-stream to approximately. 0.5 ml and transferred to a conical vial. The extracts were evaporated to just before dryness and 25 µl of DMSO was added, after which the rest of the hexane was evaporated for another 5 min-

utes. As the diluted solutions of dichlorobenze (10 mg/L), triclosan (10 mg/L), and chloroalkanes (1 g/L) were non-responsive in the first round of analysis, additional tests were
performed at higher concentrations, up to the limit of cytotoxicity. The final results of these tests at higher concentrations are included in the current final version of the report.

The DR-CALUX® bioassay is performed according to SOP Pe-BDS-004. In short, DR-CALUX® cells are grown in minimal essential medium (α-MEM, Gibco) with 10% fetal calf serum (FCS, Gibco) at a temperature of 37°C and 5% CO2.

The bioassay is performed in 96-well culture plates (Greiner). Cells are seeded in 100 µl growth medium and incubated for 24 hours until the cell layer was 90 % confluent. To each well, 100 ul α-mem containing the test compound was added, with an end concentration of 0.4% DMSO. After an additional 24 hours incubation, the medium was removed and the cells were washed once with 100 µl PBS. This PBS was also removed and 30 µl of lysismix was added. The plates were kept in the refrigerator for at least 20 minutes, after which the plates were shaken for 2 minutes at room temperature just before measuring the luminescence.

For luminescence measurement the plate was inserted in a luminometer (Lucy 2, Anthos) after which the computer program would initiate the following steps to measure the light production of each well: add 100 ul of Glowmix, measure the light production for 4 seconds, add 100 ul of 0.2 M NaOH to stop the light production.

Extract were tested in triplicates and on each plate a complete dose-response curve of 2,3,7,8-TCDD was measured as well. Curve fitting statistics were calculated using a logistic equation (SlideWrite) for each dose-response curve of TCDD after which the DR-CALUX® based TEQ of the sample was determined by interpolating the light production measured in the standard curve in the area between 1 pM and 10 pM TCDD.

2.4 Analysis of PCDD/Fs

In a selection of 3 products, responsive in the DR-CALUX® test, additional studies were done on the presence of polychlorinated dibenzodioxins (PCDDs), dibenzofurans (PCDFs), and qualitatively polychlorinated naphthalenes (PCNs). The analysis was performed by the section of Environmental and Toxicological Chemistry, Institute of Biodiversity and Ecosystem Dynamics, of the University of Amsterdam (UVA). The analytical methods used have been described in detail by de Voogt et al. (1993) and Pluijm et al. (1992). Briefly, extracts 'as received' were spiked with a mixture of ^{13}C-labeled standards of chlorinated dibenzodioxins and dibenzofurans. Extracts were cleaned up by open column chromatography using active carbon (Carbosphere, in order to separate non ortho-PCBs from PCDD/PCDF/PCN). Further clean up of the PCDD/F and PCN fraction was achieved with column chromatography on silica, loaded with H_2SO_4 and NaOH, and subsequently silica impregnated with silver nitrate. Samples were then concentrated and analysed by either GC- low resolution-EIMS (GC: HP5790, MS: HP-MSD) or GC-high resolution-EIMS (GC: HP5790, MS: Kratos Concept), using a 60 m x 0.32 mm DB-5/fused silica analytical column.

Identification of PCDD and PCDFs was based on selected m/z values (SIM), isotope cluster abundancies, and retention times of authentic standards. Identification of PCNs was based on m/z values and isotope cluster abundancies in low resolution MS. A further confirmation of identities of PCNs is their appearance in the planar (PCDD/PCDF) fraction of the clean up procedure. As no standards for PCNs were available neither retention time matching nor quantitative analysis for these compounds could be carried out. Quantitative analysis of PCDDs and PCDFs was based on response factor ratios of cold and labelled compounds.

3. Results and Discussion

3.1 DR-CALUX®

DR-CALUX® results expressed on a mass basis of pure products are indicated in Table 3.1. From the eleven products tested with the DR-CALUX® (acid-treated extracts) the highest response, exceeding the limit of quantification (LOQ) was found in chloroprene rubber, trichloroethylene and hydrochloric acid (see Table 3.1). A detectable response, at the level between the limit of detection (LOD) and LOQ, was found for 1,2-dichloroethane and perchloroethylene. For the other products the response was below the limit of detection.

Except for PER and chloroprene rubber, the results for extracts without acidic clean-up usually were higher (up to approximately 50%) than for extracts previously treated with the sulphuric acid clean-up, which indicates the presence of less stable Ah-receptor active compounds (such as e.g. PAHs). In the acid-treated extracts, it is assumed that mainly the very stable compounds contribute, such as e.g. PCDDs, PCDFs, PCBs, PCNs or brominated analogs (Behnisch et al., 2002). However as indicated by Schramm et al. (2001) in atmospheric effluent studies, the presence of PAHs (some of which may be responsive), after the acidic clean-up cannot be ruled out, depending on column type, elution solvent, and dimensions of the clean-up system. The performance of the clean-up system used in this study was tested in a separate study. (Lamoree et al., 2004). The results indicated that the most responsive higher molecular weight PAHs (with four or more aromatic rings) were effectively removed during the clean-up, but that for some responsive PAHs (benzo(b)fluoranthene, fluoranthene) the removal is not quantitative (80-90%). Based on the response in the DR-CALUX® test (Machala et al., 2001) and observed concentrations, the contribution to the response after acidic clean-up is expected to be negligible in most cases. In the further discussion we will focus mainly on the results after acidic clean-up.

The observed response for 1,2-dichloroethane (EDC) is slightly below literature data cited by Tukker (2000) indicating levels of 55 pg/g octachlorodibenzofurans and 0.06 pg/g (I-TEQs) from German studies (Heindl and Hutzinger, 1987) executed in the 1980s.

For PER, previous studies (Fiedler et al., 1994 as cited by Tukker, 2000) have indicated the presence of dioxins (mainly octachlorodibenzodioxins) in PER residues from dry cleaning operations at levels of 0.06 pg I-TEQ/g, which was attributed to impurities in dyes of clothing. The levels observed in this study for virgin PER of technical quality with the DR-CALUX® bioassay are in the same range. As no prior literature observations were found for trichloroethylene, hydrochloric acid and chloroprene rubber, these products were selected for additional analysis of PCDD/Fs.

The higher detection limits for 1,4-dichlorobenzene, triclosan and chloroparaffins are the result of the relatively low test concentrations, which had to be used for these products in this study, in order to avoid interference of cytotoxicity. The compounds were tested after acidic clean-up at maximum concentrations in the wells of the test system of 100

mg/L for Triclosan, 570 mg/L for chlorobenzene, and 5 g/L for the chloroparaffins. At these concentrations no significant DR-CALUX® response was observed.

Table 3.1 Summary of dioxin-like response (in pg DR-CALUX® based bio-TEQs per amount of product; mean values ± standard deviation) determined with DR-CALUX® including and without a sulhuric acid clean-up step [a].

Sample	Units : DR-CALUX® based 2,3,7,8 TCDD-TEQ	bio-TEQs Incl. acidic clean-up	bio-TEQs No acidic clean-up
Dichloromethane	pg TEQ/g	<0.01	<0.01
1,2-dichloroethane	pg TEQ/g	0.01 < x < 0.03 (0.02)[b]	0.02 < x < 0.04 (0.02)[b]
Perchloroethylene	pg TEQ/g	0.02 < x < 0.07 (0.04)[b]	<0.02
Trichloroethylene	pg TEQ/g	9.6 ± 0.3	18 ± 2
Allylchloride	pg TEQ/g	< 0.01	<0.02
Hydrochloric acid	pg TEQ/g	2.0 ± 0.1	2.2 ± 0.1
Monochloroacetic acid	pg TEQ/g	<0.03	<0.04
1,4-dichlorobenzene	pg TEQ/g	<200	<2000
Chloroalkanes C_{14-17}	pg TEQ/g	<20	<200
Triclosan	pg TEQ/g	<900	<9000
Chloroprene rubber	pg TEQ/g	1700 ± 83	1500 ± 61

[a] Values below limit of detection (LOD) indicated as < with numeric value of LOD; values between LOD and limit of quantitation (LOQ) indicated as < x < with numeric values of LOD and LOQ;

[b] Value between brackets is the measured value taken for further calculations.

A tentative estimation of annual loads or fluxes of DR-CALUX® based TEQs is presented in Table 3.2. For chloroprene rubber no actual usage figures were available and a preliminary surrogate value of 1000 tonnes per annum (tpa) was used to estimate the order of magnitude. According to data provided by the supplier EDC is currently used in the Netherlands only for production of vinyl-chloride (VCM) or ethyleneamines. As a preliminary estimate excluding on-site captive use of EDC (VCM)we used a figure of <80000 tpa . Total estimates for DR-CALUX® based bio-TEQ loads involved are in range of 1-2 g bio-TEQ/yr. The highest contribution is found for chloroprene rubber in the order of 1700 mg bio-TEQ/year and to a lesser extent for HCl in the order of 10-30 mg bio-TEQ/year, TRI (<5 mg bio-TEQ/yr) and EDC (<2 mg bio-TEQ/yr). The contribution for PER) is below the mg bio-TEQ/yr range. For chloroparaffins, 1,4-dichlorobenzene and triclosan the figures represent worst case estimates; the estimates are the result of the previously mentioned higher LODs for these products.

Compared to the total national emissions of dioxins (expressed in I-TEQs; RIVM, 2001) in the Netherlands in 1999 of 38 g I-TEQ/yr (water: 1-3 g; atmospheric: 35g, of which households: 28g, waste incineration: 4g and industry: 3g) the contribution from products seems only significant for chloroprene rubber (possible contribution of 1-2 g DR-CALUX® based bio-TEQ/yr) and of lesser importance for the other responsive products.

Table 3.2 Tentative estimation of annual loads of DR-CALUX® based bio-TEQs (in mg/year) via selected products.

Product	Usage tpa [a]	Estimated load in mg bio-TEQ/year	Worst case estimated load for values < LOD in mg bio-TEQ/year
Dichloromethane	4050		<0.04
1,2-dichloroethane (EDC)	<80000	<1.6 [b]	
Perchloorethylene (PER)	1500	0.06 [b]	
Trichloorethylene (TRI)	<500	<5	
Allylchloride	200		<0.003
Hydrochloric acid	<15000	<30	
1,4-dichlorobenzene	50		<10[c]
Monochloroacetic acid (MCA)	<10000		<0.3
Triclosan	3-16		<3-14[c]
Chloroalkanes C_{14-17}	1400		<28[c]
Chloroprene rubber	1000	1700	
Total		<1737	

[a] Usage outside captive use as intermediate in major production processes;

[b] Uncertain estimate based on response level between LOD and LOQ;

[c] Worst case estimate due to highest concentration tested without cytotoxicity and extrapolation to tpa.

It should be noted that the load estimates presented in Table 3.2 do not reflect actual and direct emissions to the environment as in the case of the aquatic effluent and atmospheric emission studies (Lamoree et al., 2003; Senhorst et al., 2004, Van Hattum et al., 2004). Information with respect to further applications, waste handling and leaching behaviour is required for further estimates of potential emissions. In the case of chloroprene rubber it should be noted that the Soxhlet hexane-acetone extraction probably is an overestimation of potential emissions due to leaching or weathering under natural conditions.

3.2 Presence of PCDD/Fs in responsive products

In the three most responsive products (chloroprenerubber, HCl, TRI) additional determinations were executed for the presence of PCDD and PCDF congeners, which are included in WHO/IPCC-TEF system (mammalian, UNEP, 2001; Van den Berg et al., 1998). Based on this an indication can be obtained to what extent the DR-CALUX® response after acidic clean-up can be explained from contributions of PCDD/Fs. The results for the individual PCDD and PCDF congeners are indicated in Appendix-4 and summarized in Table 3.3.

Compared to the total response determined with the DR-CALUX® (as DR-CALUX® based bio-TEQs after acidic clean-up, see Table 3.1), only a limited fraction of the dioxin-like response seems to be explained from contributions of PCDDs and PCDFs. For the different products this ranges from 5% for chloroprene rubber, 7% for TRI to 17% for HCl (calculated with non-rounded figures).

Table 3.3 Summary of concentrations of polychlorinated dibenzodioxins (PCDDs) and dibenzofurans (PCDFs) in 3 selected products responsive in the DR-CALUX® test.

Concentration	Unit	Chloroprene rubber	TRI	HCl
Total concentrations				
Σ 7-PCDDs	pg/g	943	1.7	3.8
Σ 10-PCDFs	pg/g	321	4.1	4.0
Σ 17-PCDD/Fs	pg/g	1263	5.8	7.8
2,3,7,8-TCDD equivalents	*WHO-TEQs*			
Σ 17-PCDD/Fs*	pg TEQ/g	90	0.7	0.3
TEQ contribution PCDDs	%	50%	72%	3%
TEQ contribution PCDFs	%	50%	28%	97%

* Contributions of concentrations below limit of detection not included.

Using DR-CALUX® specific relative potency values (REP) for the individual congeners (according to Benisch et al., 2002) similar figures as obtained for the fraction of the response attributable to PCDD/Fs (Chloroprene rubber 5%, TRI 6%, HCl 21%). This implies that a significant part of the DR-CALUX® response remains unexplained and attributable to other unknown compounds.

3.3 GC-MS identification study

Extracts and solutions of the products were measured with GC-MS. The chromatograms (TIC, total ion current) of the extracts and solutions are shown in Appendix-2. The mass spectra were interpreted with the AMDIS software, which applies a specific background correction technique (deconvolution, see method section) and compares the extracted spectra with the NIST reference library, which contains mass spectra of more than 10^5 compounds. For most of the products approximately several hundred to 1500 spectra were extracted by the AMDIS software, of which approximately 300-500 of could be matched (matching factor >60%) with NIST library spectra (see 3rd and 4th column in Table-3.4). Although for most products the major part of the mass spectra do not match with available library spectra in the NIST database, it is clear that in most cases the impurities are dominated by non-chlorinated compounds, similarly as with the aquatic effluent and atmospheric emission studies.

Using more stringent matching criteria (S/N ratio > 20; matching factor >70; first 3 proposed matches containing chlorine) varying but in most cases a limited number of chlorinated compounds (ranging from 8 to 65) was tentatively recognised in most of the products (see 5th column in Table 3.4). The fraction of recognised components (between brackets) and components probably containing chlorine is indicated in the 6th column as a percentage of the total area of the chromatogram. The fraction of recognised and probable chlorine containing compounds varies for most compounds between <1% (chloroparaffins, triclosan, 1,2-dichlorobenzene) and 4%. For TRI this fraction constitutes more than 50% of the area of the total chromatogram.

For the proposed tentative identifications of chlorinated compounds (7th column in Table-3.4; Appendix-1) we used more stringent criteria (with S/N ratio >50; matching factor >80%) and manual inspection of the mass spectra. An indication of the order of magnitude of the concentrations of the tentatively identified chlorinated components, present as impurities in the different products, is given in the last column of Table-3.4 and Appendix-1. The concentration intervals should be considered as indicative, as in this screening approach no separate optimisation of chromatographic conditions were made for the individual products and no prior information was available on the compound-specific response factors. Chromatograms with poor separation conditions were found for allylchloride, trichloroethylene and chloroprene rubber.

An example of the extracted mass spectrum and the matching with a library spectrum of a pentachlorophenol (as impurity in hydrochloric acid) is indicated in Figure 3.1. Further examples are given in Appendix-3.

It should be noted that the presented tentative identifications are not yet conclusive. For a complete identification mass spectrum and the retention index of the component in the sample should be compared with pure standards measured under the same conditions on the same instrument (Bobeldijk-Pastorova, 1998; EPA, 1989). As it is clear that this is beyond the scope of the OVOC project, we follow the EPA definition for 'tentative identification' when a matching with mass spectra from an external library can be demonstrated. This approach is also used in the GC-MS screening of surface waters (Barreveld, 2001; Staeb et al., 2002) in the Netherlands.

For 1,4-dichlorobenzene and triclosan much less spectra were extracted, because of the limited maximum concentrations of the test solutions (10 mg/L) that could be measured without overload of the GC column. Due to this, no other chlorinated components than the product itself could be measured for 1,2-dichlorobenzene, and only one compound for triclosan. Additional preparative separation experiments would be required to investigate if chlorinated impurities are present at lower concentrations than 1% of the product.

The chromatogram of the chloroparaffins contained far less peaks than expected for this complex mixture. The sensitivity and selectivity of the GC-MSD (LOD in the order of 0.1-1 ug/ml) is insufficient to deal with the large number (> several 1000) of $C_{14\text{-}17}$ congeners and positional isomers (with an average chlorine content of 40-63%). At the highest concentration tested (1 mg/ml), the concentrations of individual congeners probably were around or below the limits of detection. If more than 1000 congeners/isomers would have been present each individual compound would have been present at a concentration below or around 1 µg/ml. In the specific literature on chloroparaffins GC-NCI-MS is the recommended method (Santos, 2002; Tomy et al, 1998). This technique is highly specific for (multiple) halogenated compounds and up to several orders of magnitude more sensitive than the screening method (electron impact MS) applied in this study.

In the hexane-blank no chlorinated compounds were found. Traces of higher and substituted aliphates (C7-C19) and to a lesser extent an aromate (ethylbenzene) could be observed, as well as a phthalate (dibutylphtalate). These compounds were also found in

most other samples and probably originate from the GC-MS equipment (vacuum oil) or impurities in the hexane or lab materials.

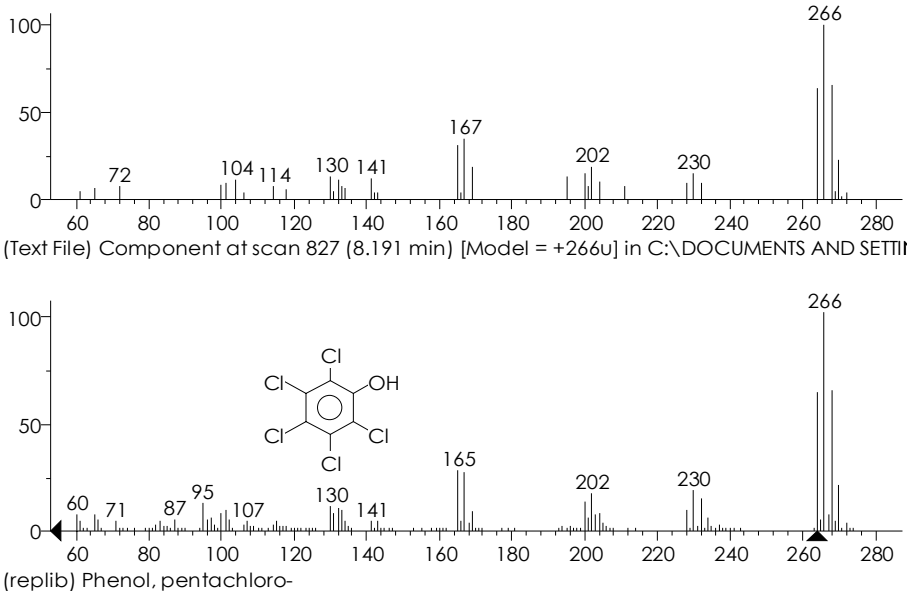


Figure 3.1. Example of extracted mass spectrum (upper figure) from hydrochloric acid (Rt = 8.19 minutes) and comparison with a reference spectrum in the NIST library for pentachlorophenol (lower figure).

Dichloromethane

Using the stringent matching criteria (S/N>50 match factor >70) no chlorinated compounds were identified. At a lower level (S/N>20) we found indications for 4 compounds present at low concentration levels of 0.1-10 ng/kg. Among these compounds 1,2-dichlorobenzene is of further interest because of the presence on OSPAR Appendix-3 list of candidate substances (OSAR, 1998). Among the non-chlorinated compounds, not present in the blank, an indication was found for the presence of a methylated PAH (2-methyl naphthalene). The qualitative analysis of non-resolved components yielded a limited number of spectra with typical chlorine clusters.

1,2-dichloroethane (EDC)

In EDC 12 different chlorinated compounds were tentatively identified with concentrations ranging from 0.01-10 µg/kg product. Components tentatively identified included: chlorinated ethers, chloroalkanes, alkylsubstituted chlorobenzenes. Several of these compounds are present on the OSPAR Appendix-3 list of candidates for selection, assessment and prioritisation. Many of the lighter components reported as impurities by the supplier are not found, probably because of evaporative losses of the sample preparation applied. Using less stringent criteria, indications were found for the presence of dichlorobutadiene.

Table 3.4 OVOC Product study - summary of extracted mass spectra of the first 8 products, matches with spectra in the NIST library, number of tentative identifications of chlorinated compounds.

Sample	Code HP-file	# Components deconvoluted by AMDIS[1]	# Deconvoluted components with match[2] in NIST library	# Components recognized by NIST possibly containing chlorine[3]	Fraction of components probably containing chlorine as % total area of chromatogram [4]	Tentatively identified chlorinated components at S/N>50 Match> 80	Concentration range of tentatively identified components in µg/kg product, unless indicated otherwise
dichloromethane	0603003.D	488	349	8	2.4% (16.9%)	0 (4)[5]	0.1-10 ng/kg
1,2-dichloroethane	0603004.D	938	405	27	0.32% (4.2%)	12	0.01-10
perchloroethylene	0603005.D	724	354	33	3.7% (59.6%)	9	0.01-100
trichlorethylene	0603007.D	1371	517	65	52.7% (76.4%)	17	0.01-1000
hydrochloric acid	0603008.D	693	406	19	0.9% (31.9%)	3	0.1-10
allylchloride	0603009.D	768	168	77	8.8% (11.4%)	8	1-500
monochloroacetic acid	0603011.D	557	271	31	3.4% (14.5%)	11	0.1-100
1,4-dichlorobenzene	2803011.D	12	6	3	0.4% (0.4%)	1 [6]	-
Chloroparaffins C_{14-17}	T070404.D	205	121 [7]	2	0.001%(42%)	-	-
triclosan	T070401.D	76	55	3	0.4%(2.6%)	2 [6]	-
chloroprene rubber	T030408.D	1552	621	12	1.5%(25.3%)	6	0.1-100 mg/kg
hexane-blank	0603012.D	51	46	0	(1.5%)	0	

[1] Deconvolution settings: component width=12; adjacent peaks substraction=2; resolution=lhigh; sensitivity=medium; shape requirements=medium; solvent tailing 32 m/z; column bleed m/z = 207; scan m/z 50-650; With these settings deconvoluted components have a signal-to-noise ratio S/N above 20;

[2] Match factor > 60;

[3] Match factor >70 or at least three of first five hits are chlorine compounds, corrected for double counts;

[4] Between brackets % of chromatogram area of compounds with match in NIST;

[5] Between brackets: matches at S/N > 20 and match factor > 70%;

[6] Including product itself;

[7] Resolution and sensitivity of MSD detection insufficient for extremely complex mixture; probably >several 10^3 congeners (Gregg et al. 1999).

Compounds that could be implied in the observed response in the DR-CALUX® test, probably are present at concentrations below the LOD of the GC-MS method.

Based on the qualitative analysis of non-resolved components, several other spectra with typical chlorine patterns were observed (indicative concentration range: 10-100 µg/kg product). Among the non-chlorinated compounds, indications were found for the presence of styrene, diphenylmethane and anthracene (S/N>50; match factor >80).

PER

Among the 9 tentative identifications of chlorinated compounds (present in concentrations ranging from 0.01 to100 µg/kg), chlorinated alkanes and alkenes dominate. Hexachlorosubstituted ethane, propene and butadiene may be relevant for further assessment based their high degree of chlorination or presence on priority lists (hexachlorobutadiene: OSPAR Appendix-3 list). A trace of the main product was found around retention time 2.1 minutes in the extract of the residue after evaporation. No relationship was found with known chlorinated compounds responsive in the DR-CALUX® test. The applied method is not sufficiently sensitive to analyse PCDD/Fs at concentration levels reported in the literature for PER (sub ng/g level; Fiedler et al., 1994). From the compounds reported by Tukker (2000b) as known impurities hexachloroethane was present in concentrations in agreement with suppliers specifications (< 5 mg/kg); the presence of chlorobenzenes could only be demonstrated with a lower level of matching of the mass spectra (S/N: 31; matching 78). Examination of unresolved components provided some information on the presence of other chlorinated compounds (number of chlorine atoms up to 7; Rt 7.4-8.2 min), with a total indicative concentration of 0.1-5 µg/kg). Among the non-chlorinated compounds butylated hydroxytoluene (BHT, an antioxidant compound), could be observed.

TRI

The largest number of tentatively identified chlorinated compounds (i.e. 17) was found in TRI, in concentrations ranging from 0.01 to 1000 µg/kg for tetra- and pentachlorinated butadienes. Further compounds included other chlorinated alkanes and alkenes, pentachlorobenzene (1-10 µg/kg) and an alkyl substituted monochlorophenol.

Chlorinated benzene, and several of the chlorinated alkanes are present on the OSPAR Appendix 3 list of candidate substances. The chlorinated butadienes and butenes may be relevant for further assessment, because of their hydrophobicity. In the EFDB-database (SRC, 1998) the Log Kow of hexachlorobutadiene is reported as 4.78 (measured value). No prior citations were found in the literature on the presence of PCDD/Fs in TRI. During the PCDD/F analysis a positive confirmation was found for the presence of a tetrachloronaphthalene compound. Some PCN congeners are considered as dioxin-likes and are responsive in the DR-CALUX® test (Behnisch et al., 2002).

Additional analysis of deconvoluted components recognised with a lower level of matching (60-80) in the RT interval 3-10 min, yielded a large number of poorly separated additional fragments with proposed identifications of tri-, tetra-, and pentachloro-substituted butadienes, hexachlorobutenes, hexachlorohexadienes, tri-, tetra-, pentachloro substituted benzenes, chloro alkyl substituted benzenes, tetra- and penta-

chloropyridines, some of which are alkyl substituted. When using an optimised chromatographic separation, it is expected that this will result in an improved deconvolution and identification. The qualitative manual search among unresolved components or components with a lower matching confirmed the presence of the typical chlorine clusters (number of chlorine atoms 4 to 8) in most spectra, with an indicative total concentration range of these components estimated at 0.1-1.5 mg/kg product. The results indicate that a multitude of chlorinated side products is present, which probably can be formed from the trichloroethylene building block, and which were only partly identified in the current screening study.

Non chlorinated compounds, not present in the blank samples that could be identified with S/N>50 and matching>80, included a.o.: alkyl-substituted benzenes and cyclopentanes, and butylated hydroxytoluene (BHT).

Allylchloride

The sample of allylchloride was reanalysed in a 10-fold dilution, as the total ion current chromatogram of the undiluted sample of allylchloride did not show sufficiently baseline separated component peaks. Five chlorinated components were tentatively identified in the first part of the chromatogram with the stringent matching criteria. The main components were 1-chloro-hexadiene (0.1-1 µg/kg, trichloropropene (1-10 µg/kg), chlorinated esters (0.1-1 µg/kg), and trichloropropane (0.1-1 µg/kg). The latter components are known by-products in the production of allylchloride. When using less stringent matching criteria (S/N>20; matching factor >70), chlorocyclohexene and chloromethylpentene were also tentatively identified. Using the chlorine cluster mass lines the qualitative manual search revealed 67 spectra, in which 1-6 similar chlorine m/z clusters could be observed. The total concentration of these components was tentatively estimated at 2-5 mg/kg. Examining non-deconvoluted sections of the chromatogram in the RT region 10-40 minutes provided indications that many more chlorinated components were present. From literature data (De Leer et al., 1985) it is known, that so-called multiple chloropropyl(di)ethers observed in sediments have been attributed to a production process in which allylchloride was used. Components containing the building blocks for these ethers, chloropropyl fragments, were found by AMDIS using the less stringent matching (S/N>20; matching >70). Although many components and structures could not be deconvoluted or identified by AMDIS due to the complexity of the chromatogram, it is clear that a large number of chlorinated by-products is present.

HCl

In hydrochloric acid chlorophenols (tri- and penta-; 0.1-1 µg/kg) and a trichloroxylene (1-10 µg/kg) were tentatively identified. Pentachlorophenol is on the Appendix-2 of the OSPAR list of chemicals for priority action; trichlorophenol is mentioned in the Appendix-3 list of candidate substances for assessment and prioritisation. HCl is set free as a by-product from various processes in the chlorine chain (such as EDC/VCM, MDI, AC/ECH, MCA, and solvent production) and sold in different technical qualities. The HCl sample used in the OVOC study is a mixture of 2 HCl-byproduct sources. Using the combined production figure for these 2 sources (15 ktpa in the period 2000-2003), would result in a load of 1.5- 15 g/yr for the (tri- and penta-) chlorophenols and 15-150 g/yr for

the trichloroxylene. Due to closure of one of the plants in 2004, the estimated loads will decrease. No prior information was found in the literature on the presence of chlorophenols in HCl (Tukker et al, 2000b) or the presence of PCDD/Fs. As stated before the DR-CALUX® response could only be partly (17-21%) attributed to the PCDD/Fs. The chlorophenols are not known to have a significant response in the DR-CALUX® test. This leaves a large part of the DR-CALUX® response unexplained. At a lower level of matching (matching: 67, S/N: 32) a mass spectrum was found (retention time 10.46) which was assigned as tetrachloronaphthalene. All major mass clusters from the library spectrum were present. Based on manual inspection it was concluded that there was a strong indication for the presence of a tetrachloronaphhtalene congener. Some of the polychlorinated naphthalenes (PCNs) are considered as dioxin-likes and are responsive in the DR-CALUX® test (Behnisch et al., 2002). Among the non-chlorinated compounds, not present in the blank samples, dibromethylbenzene and an alkylated bromophenol were observed in low quantities. The presence of the chlorinated aromatics is most likely related to one of the two selected production processes from, which the HCl originates and in which paraxylene is used in the synthesis reactions. Commercial technical HCl is produced in much larger quantities on the national scale in other production processes. However, in the prioritisation and selection of products at the start of the project, these other sources were left out for various reasons. As the organic impurities in HCl, derived as by-product, will depend to a large extent on the origin, it is clear that the results found in this study cannot be extrapolated to the Dutch HCl market. As the production of HCl from the sources in the OVOC study are only a limited fraction of the total market, future screening of other HCl qualities is of importance to obtain a more realistic estimate of possible annual loads of chlorophenols and trichloroxylene.

MCA

In MCA various alkylsubstituted chorobenzenes were tentatively identified in concentrations ranging from 0.1-10 μg/kg and from 10-100 μg/kg for 1,3,5-trichloro-2,4,6-trimethylbenzene. The proposed identification for the latter compound has a high probability (S/N = 365; matching factor 91). Using the normal identification software (PBM algorithm) of the HP Chemstation instrument the same identifications are proposed from the NIST database. The trichloro alkylsubstituted benzenes are dominant; the chlorine atoms can be bound to both the benzene ring, as well as to the alkyl chains.

The observed presence of chlorinated aromates is additional to findings of the supplier. Chlorinated aromatics were never identified by the supplier prior to this OVOC study. Information from in-company studies indicated the presence of dichloroacetic acid, non-chlorinated organic acids, low molecular weight chlorinated aliphatics. These compounds were not found in the screening approach of our study. Due to various steps in the sample preparation procedure of the OVOC project (L/L extractions, evaporation steps), which is targeted on non-volatile hydrophobic compounds, the recovery for such compounds is expected to be limited. Additionally, it should be noted that the NIST mass spectral database, although it may cover over 100.000 compounds, may not cover sufficiently mass spectra of unintended by-products which have no technical or scientific interest. Such specific mass spectra may be present within companies. In order to rule-out the possibility of reaction of MCA with aromatic impurities in the hexane used for the extraction, as a source for the alkylsubstituted chlorobenzenes, the extractions were

repeated with dichloromethane (DCM), the solvent used in the studies conducted by the supplier, and with a mixture of DCM and pentane (1/1 v/v). Both additional extraction runs yielded similar chlorinated aromatic compounds as in the hexane extractions. Recent studies by the supplier on new samples taken in 2004 confirmed the presence of chlorinated aromatic compounds.

In the qualitative analysis of non-identified components or components with a matching between 60 and 80 (S/N>50), plots of mass lines in the range m/z=72-78 (corresponding to $C6Hy^+$ fragments of benzene) were used to scan for other aromatic compounds in the chromatograms. After examination of the presence of typical chlorine clusters in the mass spectra, the number of chlorine atoms was investigated. In this way, 39 different components in the retention time interval 6.1 – 8.9 minutes were found, which contained both the typical fragment of the benzene core and the typical mass clusters of 2, 3 or 4 chorine atoms in the molecule. Among the components identified with a lower matching (>60 and <80) many (15-20) different other chlorinated alkyl substituted benzenes and related compounds (RT interval 6.1-8.5) are proposed from the NIST database, sometimes with a poor separation. Improving the chromatographic separation (a generic screening program was used in this study) probably would result in more deconvoluted and recognised components. The findings seem to corroborate that more chlorinated aromatic compounds are present, than the proposed tentative identifications which resulted from the AMDIS/NIST analysis (with S/N>50 and matching>80). The concentration level of these additional compounds was estimated tentatively between 0.1 – 1 mg/kg.

Chloroprene rubber

In the Soxhlet-extracts of chloroprene rubber 6 chlorinated compounds were tentatively identified (S/N>50, matching >80), in concentrations ranging from 0.1-1 mg/kg (trichloromethylpropene and 2 chlorinated alkylsubstituted benzenes), 1-10 mg/kg (dichloroethenylcyclohexenes), and 10-100 mg/kg for dichlorocyclooctadiene. Among the non-chlorinated compounds tentatively identified were various PAHs (naphthalene, fluoranthene, benzo(k)fluoranthene, pyrene, chrysene, indeno(1,2,3-cd)pyrene, coronene), a sulphur containing aromatic compound (3,4-dimethylthiophene), a cluster of phthalates (mainly di-n-octylphthalate, large broad cluster of peaks between Rt 30-33 minutes), butylated hydroxytoluene and dioctyldiphenylamine (antioxidants). When using less stringent matching criteria (S/N>30; matching factor >70) indications were found for the presence of 1,1-dichloro-1,2-butadiene. In the study of Fuh and Wang (1998) where pyrolysis GC-MS was applied similar and partly matching compounds were identified, that were also found in the OVOC study: chlorobutadiene (monomer), dichloroethenylcyclohexene (dimer), chloromethylbenzene, chlorobenzene.

The measured concentrations of PCDD/Fs only partly (5-7%) explain the observed DR-CALUX® response. None of the other chlorinated compounds encountered is known to be responsive in the DR-CALUX® test. Among the non-chlorinated compounds, the PAHs might be of relevance, as some are known to be responsive in the DR-CALUX® test (Machala et al., 2001). The total concentration of the PAHs present is estimated at approximately 50 mg/kg (range 10-100 mg/kg). Among the PAHs identified in the chloroprene rubber extract (without acidic clean-up), only the most responsive PAHs

(with a relative potency, REP, larger than 10^{-6} compared to 2,3,7,8-TCDD) might have a significant contribution, if present in relevant concentrations. Possible candidates analysed in the raw extract were: benzo(k)fluoranthene (with a REP of $1.6.10^{-3}$) and indeno(1,2,3,cd)pyrene (with a REP of $3.\ 10^{-4}$). As these compounds are expected to be removed during the acidic clean-up (Lamoree et al., 2004), the DR-CALUX® response probably cannot be explained from contributions of these PAHs. Additional possible but less likely explanations could be, overloading of the acid clean-up column, or the presence of unidentified and more responsive substituted PAHs. The presence of the identified chlorinated compounds and non-chlorinated compounds is in agreement with available literature on the complex composition of the mixtures of additives used for the formulation of chloroprene rubber (ECB, 2003; TGD-Part 4 Emission scenario documents) and confidential data provided by the supplier. The PAHs originate most likely from carbon black, one of the major constituents (up to approximately 30%) of technical chloroprene rubber formulations.

As chloroprene rubber is also applied in consumer products, such as wetsuits, the findings of this study may possibly indicate a not previously known pathway for exposure to DR-CALUX® responsive compounds. However, additional information on the causative agents, representative exposure conditions, and on differences in technical qualities of chloroprene rubber is required before further conclusions on the significance in this respect can be drawn.

Chloroprene rubber was one of the two products selected as a 'wildcard' and had the highest concentrations of PCDD/Fs and chlorinated impurities. This may indicate that current regulatory assessment schemes may not be completely comprehensive, and that e.g. chlorinated impurities in imported products not considered in the chlorine balance studies (e.g. rubber products, pharmaceuticals and personal care products) may need further attention in this respect.

Summary of identifications

In Table 3.5 the total concentrations are summarized of classes of chlorinated compounds identified with the AMDIS/NIST procedure (*tentatively identified* compounds) and based on the manual examination. This latter group of compounds will be referred to as *indicatively characterized* components. The ranges reported for each product for the tentatively identified compounds are based on a summation of the lower and upper values of the concentration ranges of the tentatively identified compounds mentioned in Annex-1. For the indicatively characterized components, rounded order of magnitude ranges are reported as either 0.1-1 intervals of powers of 10 (for values within 5-95 % of the upper interval boundary) or 0.5-5 intervals (for values within ± 5% of the interval boundary). This convention of rounded ranges was used in this report, because of the large uncertainties involved in the quantification. For comparison the summed amounts of the point estimates are given in brackets.

The highest concentrations of total chlorinated impurities (Table 3.6, sum of tentatively identified and indicatively characterized compounds) seem to be present in the chloroprene rubber extract (20-200 mg/kg), followed by allylchloride (1-10 mg/kg), TRI (0.3-3 mg/kg), and MCA (0.1 – 1.1 mg/kg) with concentrations in the low and sub mg/kg region. Sub mg/kg concentrations were found in EDC and HCl (0.01-0.1 mg/kg). In PER

the concentrations are below 0.03 mg/kg. The lowest concentrations were found in DCM in the low ppb range (< 0.005 mg/kg).

Potential worst-case annual loads have been indicated in Table 3.5, with contributions in the kg/year range for chloroprene rubber (20-200 kg/yr), MCA (<1-11 kg/yr), EDC (<0.8-8 kg/yr), allylchloride (0.2-2 kg/yr), TRI (<0.2-1.5 kg/yr), and HCl (<0.1-1.5 kg/yr). These worst-case annual loads should explicitly be seen as contributions for situations with open and dispersive use patterns. Further detailed information on the applications, final fate and waste-handling practices is required to provide a more realistic estimate of the actual emissions.

Table 3.5 Tentative estimate of worst-case annual loads of chlorinated impurities in products.

Product	Usage [a] t/yr	Concentration chlorinated impurities range (mg/kg) [b]	Worst case annual load chlorinated impurities kg/yr
Chloroprene rubber	1000	20-200	20-200
TRI	<500	0.3-3	< 0.2-1.5
MCA	<10000	0.1-1.1	<1-11
AC	200	1.1-11	0.2-2.2
EDC	<80000	0.01-0.1	<0.8-8
PER	1477	0.003-0.03	0.004-0.04
HCl	<15000	0.01-0.1	<0.1-1.5
DCM	4050	<0.005	<0.02

[a]: See remarks made for Table 3.1;
[b]: Total of tentatively identified and indicatively characterized compounds in Table 3.5.

Table 3.6 Summary of estimated concentration ranges[a] of chlorinated compounds tentatively identified using AMDIS or characterized via manual examination of unresolved deconvoluted components.

Product	Content tentatively identified chlorinated compounds AMDIS (mg/kg)[b]	Content indicatively characterized chlorinated compounds from manual examination (mg/kg)[c]	Degree of chlorination	Character of chlorinated compounds
Chloroprene rubber	10-100 (24)	10-100 (67)	1,2,3	Benzene, cyclohexene, butadiene, cyclooctadiene
TRI	0.2-2 (1.1)	0.1-1 (0.3)	1-6 (8)	Butadienes, butenes, hexadienes, benzenes, naphthalene
AC	0.1-1 (0.24)	1-10 (>5.1)[d]	1,3,4,5,6	Propene, propane, pentene, hexene, hexadiene
MCA	0.01-0.1 (0.04)	0.1-1 (0.54)	2,3,4	Alkylated benzene, xylene
EDC	0.002-0.02 (0.005)	0.01-0.1 (0.06)	1,2,3	Esters, ethers, benzene groups
PER	0.002-0.02(0.004)	0.001-0.01 (0.003)	1,3,5,6	Alkanes, alkenes
HCl	0.001-0.01 (0.005)	0.01-0.1 (0.02)	2,3,4,5,7	Benzene, phenol, xylene
DCM	<0.00003[e]	<0.005 (0.001)		Propene, benzene

[a]: As rounded order of magnitude intervals, (uncertain) point-estimates between brackets ;
[b]: determined with SN>50 and matching>80, summation of ranges indicated in Annex-1;
[c]: method described in text sections 2.2 and 3.3;
[d]: 67 compounds at retention time interval RT <10; multiple unresolved chlorinated compounds present in RT region 10-40 min. not further quantified;
[e]: determined with SN>20 and matching>70.

4. Conclusions

- A selection of 11 products was screened for the presence of impurities with a dioxin-like response (DR-CALUX® assay) and the presence of unexpected chlorinated compounds (GC-MS). Three products (chloroprene rubber, hydrochloric acid and TRI) were responsive at well quantifiable levels (1-1700 pg bio-TEQs/g); in two products (1,2-dichloroethane, perchloroethylene) a response was detected at levels between LOD and LOQ (0.01-0.07 pg DR-CALUX® TEQ/g);
- In the three most responsive products PCDDs and PCDFs were analysed with HRGCMS and observed in concentrations ranging from 0.3 pg WHO-TEQs/g (HCl) to 0.7 pg TEQ/g (TRI) and 90 pg TEQ/g (chloroprene rubber). In TRI the presence of a tetrachloronaphthalene was confirmed with HRGCMS. Only a limited fraction of the bioassay-based dioxin-like response seems to be attributable to PCDD/Fs, ranging from 5% (chloroprene rubber) to 17-21% (HCl);
- Tentative estimations of annual loads or fluxes of DR-CALUX® based dioxin equivalents (bio-TEQ after acidic clean-up), due to the national usage of the selected products, indicated possible contributions in the order 1-2 g bio-TEQ per year for chloroprene rubber, approximately 0.01-0.03 g bio-TEQ/year for HCl, <0.005 g bio-TEQ/year for TRI, and <0.002 g bio-TEQ/yr for EDC and PER. Compared to the total national emissions of dioxins, a possible contribution from products only seems relevant for chloroprene rubber;
- The GC-MS screening studies were successful for 8 of the 11 products and demonstrated the complexity of the mixtures of impurities present and the dominance of non-chlorinated constituents in most samples. In the products several hundred to more than 1000 distinct mass spectra were found, of which approximately 40-75% could be matched with library spectra from the NIST database, of which a limited number (8-65) contained chlorine with a high probability (S/N>20; matching >70), and of which only a few (3-17) could be tentatively identified (S/N>50; matching >80) at approximate concentration levels of 0.1-100 mg/kg (Soxhlet-extractable) compounds for chloroprene rubber and 0.00001-1 mg/kg for 7 other products;
- Further manual examination of non-identified components revealed the presence of multiple chlorinated compounds, especially in TRI, allylchloride, MCA, and chloroprene rubber. In many cases homologous series of related compounds with a varying degree of chlorination were found, which except for MCA could partly be attributed to the specific production process. Tentative estimates of total annual loads of chlorinated impurities in the products varied from <0.02 kg/yr for DCM, <0.04 kg/yr for PER, <0.1-1.5 kg for TRI and HCL, 0.2-2 kg for allylchloride, <0.8-8 kg for EDC, <1-11 kg for MCA, and 20-200 kg for chloroprene rubber;
- Among the chlorinated components identified relatively light components seemed to dominate in most products. Specific compound classes that need to be further addressed in Phase-3 of the OVOC project included: chlorinated phenols (in TRI and HCl), chlorinated (alkylsubstituted) benzenes (in 1,2-dichloroethane, dichloromethane, HCl, MCA and chloroprene rubber), and chlorinated alkanes or alkenes (C4 and higher in PER, TRI and chloroprene rubber). Some of these compounds have PBT properties and are present on OSPAR lists of priority chemicals;

- Due to problems related to chromatographic separation, selectivity or sensitivity the GC-MS based screening approach was not successful for 1,4-dichlorobenzene, triclosan, and chloroparaffins. Additional separation studies and application of HRGC-NCI-MS would be required to analyse for chlorinated impurities at levels below 1%, but this was beyond the scope of the screening study;
- The DR-CALUX® response in chloroprene rubber, TRI, and HCl could only be partly (5-21%) attributed in this study to the classical dioxins (PCDD/Fs). This leaves a large part attributable to unknown compounds, possibly a mixture of dioxin-likes and non-dioxin likes. For TRI the confirmed presence of a tetrachloronaphthalene may point to a possible contribution from this class of compounds. For HCl there was an indication of the presence of a tetrachlorinated naphthalene. As no information is available on concentration levels no conclusions can be drawn on the contribution to the DR-CALUX® response. No other causal links could be made with other chlorinated compounds identified in the GC-MS screening study. The identified PAHs in the chloroprene rubber formulation (which contained up to 30% carbon black), some of which are known to be responsive, are not likely to have contributed to the DR-CALUX® response, because of the removal during the acidic clean-up, but contributions from other unknown substituted PAHs, which could not be analysed in this screening study and which might be responsive and resistant to the acidic clean-up, cannot be ruled out fully as a possible explanation;
- The fact that the most responsive product (chloroprene rubber) was one of the two products selected as 'wildcard' indicates that the prioritisation procedure in the OVOC project was not comprehensive. Chlorinated impurities in imported products may need further attention.

References

Barreveld, H.L., Berbee, R.P.M. & Ferdinandy, M.M.A. (2001). *Vergeten stoffen in Nederlands oppervlaktewater*. RIZA rapport nr. 2001.020. RIZA, Lelystad.

Belfroid, A.C., Stroomberg, G.J., Letcher, R.J., Stam, G.J. & Van Hattum, B. (2000). *Chemical and bioscreening in search of toxic chlorine compounds in waste water. Integrative report on the results of the pilot study OVOC*. IVM Report (W-00/10). Vrije Universiteit, Amsterdam.

Benisch, P. et al. (2002). Screening of Dioxin-Like Toxicity Equivalents for Various Matrices with Wildtype and Recombinant Rat Hepatoma H4IIE Cells. *Toxicological sciences, 69*, 125-130.

Bobeldijk-Pastorova, I. (1998). *Identification criteria for GC-MS analysis if environmental contaminants in different matrices*. Report nr., KOA 98.222. KIWA, Nieuwegein. (in Dutch).

De Koning, A. & Tukker, A. (2004). *Literature study on the importance of current anthropogenic PCDD/PCDF emissions in the Netherlands.* CML-IE Working Paper 2004.001. Centre of Environmental Science, Leiden University, Leiden.

De Leer, E.W.B. (1985). The identification of highly chlorinated ethers and diethers in river sediment near an epichlorohydrin plant. *Water Res., 19*, 1411-1419.

De Voogt P., Van Velzen, M.J.M. & Leonards, P.E.G. (1993). PCB metabolites, chlorinated dioxins and furans in tissues of mustelids from The Netherlands. *Organohalogen Compounds, 14*, 105-108.

Dugan, S. (2000). Comparison of gas chromatography–pulsed flame photometric detection–mass spectrometry, automated mass spectral deconvolution and identification system and gas chromatography–tandem mass spectrometry as tools for trace level detection and identification. *Journal of Chromatography A*, *868*, 229–247.

ECETOC (1999). *Joint Assessment of Commodity Chemicals No. 39. Tetrachloroethylene*. ECETOC, Brussels, Belgium.

EPA (1989). *Semi-volatile organic compounds by isotope dilution GC-MS*. EPA method 1625, Revision C, June 1989.

ECB (2003). Technical Guidance Document on Risk Assessment.- Part IV. Report nr. EUR 20418 EN/4. Environmental Chemical Bureau. European Commission. Joint Research Centre, ISPRA It. Chapter-7. Emission Scenario Documents, 177-203.

Fiedler, H. (1994). Sources of PCDD/PCDF and Impact on the Environment. *Organohalogen Compounds, 20*, 229.

Fuh, M.S.& Wang, G. (1998). Quantitative analysis of nitrile rubber/chloroprene rubber by pyrolysis gas chromatography mass spectrometry. *Analytica Chimica Acta, 371,* 89-96.

Heindl, A. & Hutzinger, O. (1987). Search for industrial sources of PCDD/PCDF. III. Short Chain Chlorinated Hydrocarbonds. *Chemosphere, 16*, 1949-1597.

ICI (1994). Written communication, June 1994.

Kleijn, R., Elshkaki, A., De Koning, A. & Tukker, A. (2001a). *Literature study on unknown organochlorine compounds in the environment from anthropogenic sources*. CML-SSP Working Paper 2001.001. Centre of Environmental Science, Leiden University, Leiden (NL).

Kleijn, R., Elshkaki, A., De Koning, A. & Tukker, A. (2001b). *Literature study on degradation products of known emissions*. CML-SSP Working Paper 2001.001. Centre of Environmental Science, Leiden University, Leiden (NL).

Lamoree, M.H., Sanderson, T., Senhorst, H. & Van Hattum, B. (2003). *Chlorinated Micropollutants in Aquatic Effluents. Part-3 Refined fractionation and primary TIE studies*. IVM Report (E-03/02). Vrije Universiteit, Amsterdam.

Machala, M., Vondracek, J., Blaha, L., Ciganek, M. & Neca, J.V. (2001). Aryl hydrocarbon receptor-mediated activity of mutagenic polycyclic aromatic hydrocarbons determined using in vitro reporter gene assay. *Mutat Res, 497*, 49-62.

Mallard, W.G. & Reed, J. (1997). *Automated Mass Spectral Deconvolution & Identification System AMDIS — USER GUIDE*. U.S. Department of Commerce Technology Administration, National Institute of Standards and Technology (NIST) Gaithersburg, MD 20899, USA.

McLafferty, F.W. & Turecek, F. (1993). In: *Interpretation of mass spectra*, 4th edition, 283-291.

Meylan, W. & Howard, P. (1999b). *User's guide for HENRYWINc Version 3, Henry's law constant pro-gram for Microsoft Windows*. Syracuse Research Corporation, North Syracuse (NY).

Meylan, W. & Howard, P. (1999c). *User's guide for KOWWINc Version 1.6, Log Octanol-Water Partition Coefficient Estimation Program for Microsoft Windows*. Syracuse Research Corporation, North Syracuse (NY).

Murk et al (1998). Application of biomarkers for exposure and effect of polyhalogenated aromatic hydrocarbons in naturally exposed European otters (Lutra lutra*). Environ. Toxicol, Pharm., 6*(2), 91-102.

NATO/CCMS (1988). *Scientific basis for the development of the International Toxicity Equivalency Factor (I-TEF) method of risk assessment for complex mixtures of dioxins and related compounds*. North Atlantic Treaty Organisation, Brussels.

OSPAR (1998). *OSPAR Strategy with regard to Hazardous Substances*. Appendix-3. List of Candidate Substances.

Pemyna, G.M., Venkataraghovan, R., Dayringen, H.E. & McLafferty, F.W. (1976). *Anal. Chem.*, *48,* 1362-1368.

Pluim, H.J., Slot, P.C., Olie, K., Van der Slikke, J.W. & Kopper, J.G. (1992). Diurnal variations in concentrations of PCDDs and PCDFs in human milk. *Chemosphere, 25*, 307-311.

RIVM (2001). *Miliebalans 2001*. Rijksinstituut voor Volksgezondheid en Milieu, Bilthoven.

Santos, F.J. & Galceran, M.T. (2002). The application of gas chromatographt to environmental analysis. *Trends in Anal. Chem., 21,* 672-684.

Schramm, K.A., Klimm a, C., Hofmaier b, A. & Kettrup, A. (2001). Comparison of dioxin-like-response in vitro and chemical analysis of emissions and materials. *Chemosphere, 42,* 551-557.

Senhorst, H., Lamoree, M.H., Besselink, H., Sanderson, T. & Van Hattum, B. (2004). *Chlorinated Micropollutants in Aquatic Effluents. Part-4 In-pant TIE studies (Workpackage 9)*. IVM Report (E-04/03). Vrije Universiteit, Amsterdam.

SRC (1998). *Using Syracuse research corporation's environmental fate databases*. Syracuse Research Corporation, North Syracuse NY (USA).

Staeb, J.A., Epema, O.J., Van Duijn, P., Steevens, J., Klap, V.A. & Freriks, I.L. (2002) Automated storage of gas chromatography-mass spectrometry data in a relational database to facilitate compound screening and identification. *Journal of Chromatography A, 974,* 223-230.

Tomy, G. et al. (1998). Interlaboratory study on quantitative methods of analysis of C_{10}-C_{13} polychloro-n-alkanes. *Anal. Chem., 71*, 446-451.

Tukker, A. (2000a). *Chlorinated micropollutants: priority setting for the sampling campaign in the main research phase. Report of Task 3.1 and 3.2 of the Preliminary Investigation of the Chlorine Micropollutant study*. Report No. 00-34. TNO-STB, Delft (NL).

Tukker, A. (2000b). *Chlorinated micropollutants: Literature search on emissions. Backgrounds to the priority setting for the main research phase*. TNO-STB, Delft (NL).

Tukker, A., Kleijn, R. & Smeets, W.R.W. (1997). Chlorine in the Netherlands, part III – Risk management in uncertainty for chlorine. *J. Industrial Ecology, 1*, 91-110.

UNEP (2001). *Standardized Toolkit for Identification and Quantification of Dioxin and Furan Releases*. Draft. UNEP Chemicals Switzerland.

Van den Berg, M., Birnbaum, L., Bosveld, B. T. C., Brunstrom, B., Cook, P., Feely, M. & Giesy, J. P. (1998). Toxic equivalency factors (TEFs) for PCBs, PCDDs and PCDFs for humans and wildlife. *Environ. Health Perspect., 106*, 775-792.

Van Hattum, B., Pols, H., Van den Berg, M., Seinen, W., Brouwer, A., Tukker, A., Kleijn, R. & Wegener, J.W.M. (1998). *Proposal Research Programme Chlorine Chain Follow-Up Studies (OVOC)*. Vrije Universiteit, Amsterdam. (In Dutch, English translation available).

Van Hattum, B., Tukker, A., Senhorst, H., van den Berg, M., Belfroid, A. & Lamoree, M.H. (2000). *Research Programme Chlorine Chain Follow-up Studies (OVOC)*. Work plan Main Studies – Phase 2. IVM Report (W-00/27). Vrije Universiteit, Amsterdam (NL), pp. 58.

Appendix I. Components tentatively identified and indicative concentration ranges

Indicated are the tentative identifications from the GC-MS analysis and automated comparison (AMDIS) with the NIST database of mass spectra. Indicated are assigned components (with CAS or EPA nr), retention time of modelled component, S/N ratio (reconstructed ion current), weighted matching (forward) and calculated concentration range. In case of components occurring at different retention times, isomeric structures of the assigned component are likely to be involved, which are not included in NIST. In this case the assignments should be considered as indicative for the group of isomers (e.g. trichlorodimethylbenzene isomer, instead of trichloro-1,3-dimethylbenzene).

1,2-dichloroethane

CAS nr EPA-nr	Name	R.T. min.	S/N ratio	Weighted Matching %		Concentration ug/lg interval
97972	Ethane, 2-chloro-1,1-	2.087	198	95		0.1-1
638288	Hexane, 2-chloro-	2.1	103	90		0.01-0.1
116541	Acetic acid, dichloro-, methyl ester	2.402	81	87		<0.01
2568301	1,3-Dioxolane, 2-	3.151	210	90		0.1-1
111444	s-Dichloroethyl ether	4.123	116	94		0.01-0.1
EPA-216775	Methyl E-2,3-	4.732	660	80		1-10
111911	Methane, bis(2-chloroethoxy)-	5.382	742	95		1-10
54411120	Benzene, (2-chloro-2-butenyl)-	5.697	292	88		0.1-1*
EPA-161079	Cyclopropane, 1-chloro-1-methyl-2-	5.921	415	92		0.1-1*
1123848	Benzene, 1,4-dichloro-2-ethenyl-	6.259	102	84		0.01-0.1
5409756	Ethane, 2-chloro-1,1-bis(2-chloroethoxy)-	6.671	195	90		0.01-0.1
2436739	Acetic acid, (4-chloro-2-methylphenoxy)-, methyl	7.341	150	83		0.01-0.1

*including contributions of component at adjacent R.T

dichloromethane

CAS nr EPA-nr	Name	R.T. min.	S/N ratio	Weighted Matching %		Concentration ng/kg interval
127184	Tetrachloroethylene	2.142	22	90		0.1-1
2233003	1-Propene, 3,3,3-trichloro-	3.716	23	80		0.1-1
95501	Benzene, 1,2-dichloro-	4.374	42	90		1-10
7325840	Silane, trichlorodocosyl-	8.455	86	71		1-10

monochloroacetic acid

CAS nr EPA-nr	Name	R.T. min.	S/N ratio	Weighted Matching %		Concentration ug/kg interval
2077465	Benzene, 1,2,4-trichloro-3-methyl-	6.131	207	93		0.1-1
54965194	Benzene, trichloroethyl-	6.519	99	89		0.1-1
54965207	Benzene, trichloro-1,3-dimethyl-	6.619	155	91		0.1-1*
54965014	Benzene, 1,2-dichloro-4-(1-chloroethyl)-	6.75	335	84		1-10
74421515	Benzene, trichloro[(dichlorophenyl)methoxy]-	6.877	68	81		0.1-1
3955268	Benzene, 1,2,4-trichloro-5-(chloromethyl)-	6.952	294	83		1-10*
54965207	Benzene, trichloro-1,3-dimethyl-	7.034	146	81		
5324685	Benzene, 1,3,5-trichloro-2,4,6-trimethyl-	7.236	364	91		10-100
6298722	2,5-Bis(chloromethyl)-p-xylene	7.258	212	80		0.1-1*
877098	Benzene, 1,2,3,5-tetrachloro-4,6-dimethyl-	7.574	592	84		1-10
54833135	Benzene, 1,2,4-trichlorodiethyl-	7.69	452	84		1-10

*including contributions of component at adjacent or nearby RT intervals

hydrochloric acid

CAS nr EPA-nr	Name	R.T. min.	S/N ratio	Weighted Matching %		Concentration ug/kg interval
88062	Phenol, 2,4,6-trichloro-	6.424	107	92		0.1-1
54965207	Benzene, trichloro-1,3-	7.718	537	85		1-10
87865	Phenol, pentachloro-	8.191	118	82		0.1-1

perchloroethylene

CAS nr EPA-nr	Name	R.T. min.	S/N ratio	Weighted Matching %		Concentration ug/kg interval
127184	Tetrachloroethylene (=PER in residue after evaporation)	2.04	90	86		10-100*
598992	Acetic acid, trichloro-, methyl ester	3.309	67	94		0.01-0.1
96184	Propane, 1,2,3-trichloro-	3.52	93	94		0.01-0.1
1871585	Propane, 1,2,3-trichloro-2-methyl-	3.866	60	84		0.01-0.1
EPA-146756	Methyl trichloropropenoate	4.616	218	88		0.1-1
67721	Ethane, hexachloro-	4.818	767	81		1-10
594650	Acetamide, 2,2,2-trichloro-	5.362	154	81		0.1-1
1888717	1-Propene, 1,1,2,3,3,3-hexachloro-	5.627	789	91		1-10
87683	1,3-Butadiene, 1,1,2,3,4,4-hexachloro-	5.653	88	88		0.1-1*

*including contributions of component at adjacent R.T intervals

allylchloride

CAS nr EPA-nr	Name	R.T. min.	S/N ratio	Weighted Matching %		Concentration ug/kg interval
10061026	1-Propene, -dichloro	2.372	207	96		10-100
13116579	1-Propene, 1,2,3-trichloro-*	3.13-3.71	52-83	86-88		1-10
EPA-131849	Chloroacetic acid, 1-methyl	3.834	79	88		1-10
96231	2-Propanol, 1,3-dichloro-*	4.00-4.17	88-174	81-93		10-100
96184	Propane, 1,2,3-trichloro-	4.251	78	92		1-10
691930	Propanoic acid, 3-chloro-, 1-methylethyl	4.58,4.62, 4.69	73-378	81-92		10-100
96242	1,2-Propanediol, 3-chloro-	4.651	179	89		10-100
27990745	1-Pentene, 5-chloro-4-(chloromethyl)-	5.02-5.05	318-361	86		50-500

*including contributions of component at adjacent and other RT

trichloroethylene

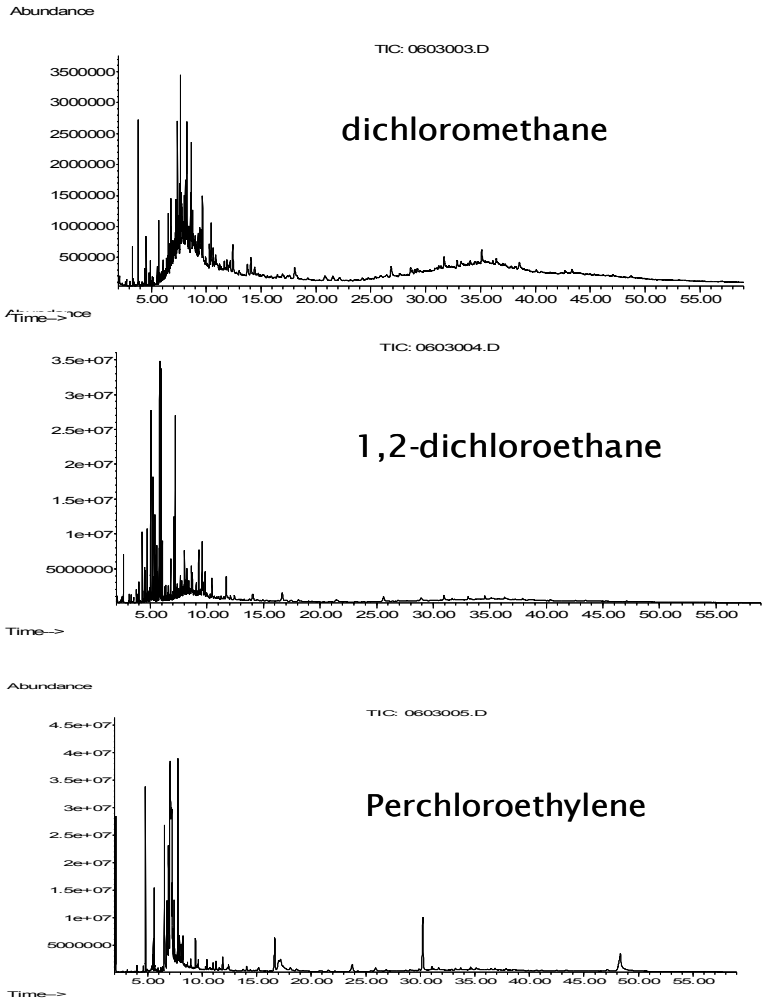
CAS nr EPA-nr	Name	R.T. min.	S/N ratio	Weighted Matching %		Concentration ug/kg interval
2346818	3-Chlorohexane	2.052	91	83		0.01-0.1
127184	Tetrachloroethylene	2.068	109	96		0.1-1
116541	Acetic acid, dichloro-, methyl ester	2.351	313	89		0.1-1
13116579	1-Propene, 1,2,3-trichloro-, (Z)-	2.818	67	92		0.01-0.1
598992	Acetic acid, trichloro-, methyl ester	3.286	51	89		0.01-0.1
79345	Ethane, 1,1,2,2-tetrachloro-	3.428	81	84		0.01-0.1
EPA-145923	Methyl 3,3-dichloropropenoate	3.573	199	91		0.1-1
76017	Ethane, pentachloro-	4.039	808	82		1-10
95501	Benzene, 1,2-dichloro-	4.359	135	90		0.1-1
20589859	1-Propene, 1,2,3,3-tetrachloro-	4.554	146	86		0.1-1
42769380	1,3-Butadiene, 1,1,3,4-tetrachloro-	4.633	210	84		1-10*
	idem broad peaks	4.63-4.98				100-1000
55880778	1,3-Butadiene, pentachloro-	5.179	623	85		10-100
	idem broad peaks	5.17-5.58				100-1000
1888717	1-Propene, 1,1,2,3,3,3-hexachloro-	5.708	576	85		1-10
87683	1,3-Butadiene, 1,1,2,3,4,4-hexachloro-	5.726	1224	89		10-100
56631017	Butene, hexachloro-	6.416	222	80		0.1-1*
34973392	1-Butene, 1,1,3,3,4,4-hexachloro-	6.425	470	85		
34973392	1-Butene, 1,1,3,3,4,4-hexachloro-	6.461	568	83		
89689	Phenol, 4-chloro-5-methyl-2-(1-methylethyl)-	7.148	1085	90		1-10
608935	Benzene, pentachloro-	7.273	1151	89		1-10

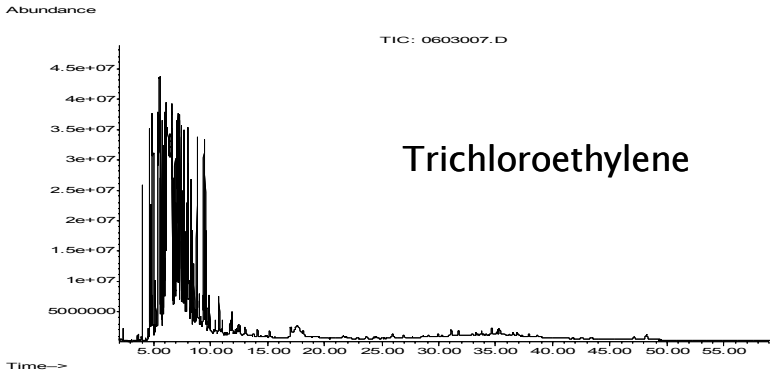
*including contributions of component at adjacent R.T intervals

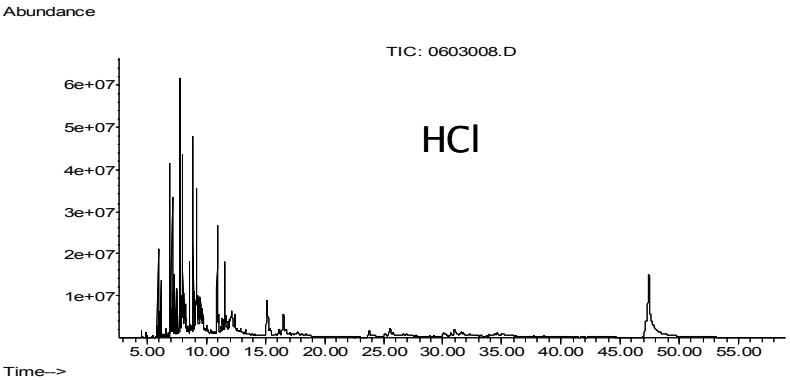
neoprene

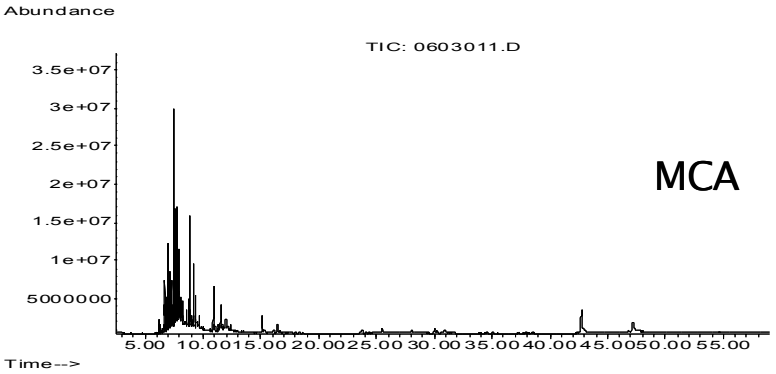
CAS nr EPA-nr	Name	R.T. min.	S/N ratio	Weighted Matching %		Concentration mg/kg interval
4749273	1-Propene, 3,3,3-trichloro-2-	4.751	74	81		0.1-1
1073672	Benzene, 1-chloro-4-ethenyl-	5.635	174	94		0.1-1
104825	Benzene, 1-(chloromethyl)-4-methyl-	5.987	154	84		0.1-1
13547074	Cyclohexene, 1-chloro-5-(1-chloroethenyl)-	6.599	562	93		1-10
13547063	Cyclohexene, 1-chloro-4-(1-chloroethenyl)-	6.641	655	93		1-10
29480420	1,5-Cyclooctadiene, 1,6-dichloro-	7.195	499	90		10-100

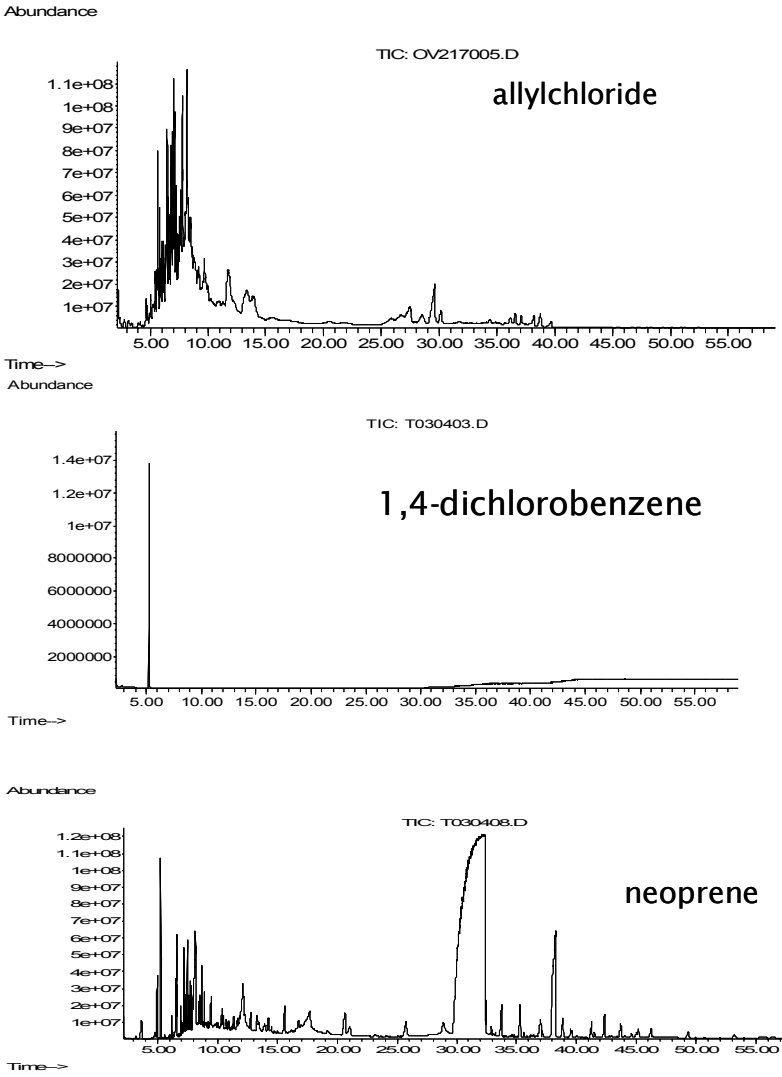
Appendix II. GC-MS chromatograms of total ion current (TIC)

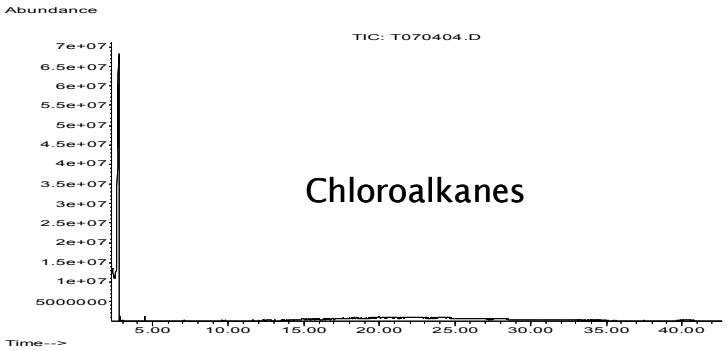


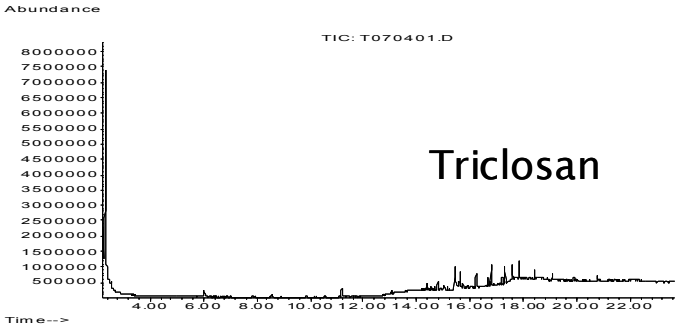

Abundance
TIC: 0603007.D
4.5e+07
4e+07
3.5e+07
3e+07
2.5e+07
2e+07
1.5e+07
1e+07
5000000
5.00 10.00 15.00 20.00 25.00 30.00 35.00 40.00 45.00 50.00 55.00
Time-->
Trichloroethylene


Abundance
TIC: 0603008.D
6e+07
5e+07
4e+07
3e+07
2e+07
1e+07
5.00 10.00 15.00 20.00 25.00 30.00 35.00 40.00 45.00 50.00 55.00
Time-->
HCl


Abundance
TIC: 0603011.D
3.5e+07
3e+07
2.5e+07
2e+07
1.5e+07
1e+07
5000000
5.00 10.00 15.00 20.00 25.00 30.00 35.00 40.00 45.00 50.00 55.00
Time-->
MCA


Abundance
TIC: OV217005.D
allylchloride
1.1e+08
1e+08
9e+07
8e+07
7e+07
6e+07
5e+07
4e+07
3e+07
2e+07
1e+07
5.00 10.00 15.00 20.00 25.00 30.00 35.00 40.00 45.00 50.00 55.00
Time-->
Abundance
TIC: T030403.D
1,4-dichlorobenzene
1.4e+07
1.2e+07
1e+07
8000000
6000000
4000000
2000000
5.00 10.00 15.00 20.00 25.00 30.00 35.00 40.00 45.00 50.00 55.00
Time-->
Abundance
TIC: T030408.D
neoprene
1.2e+08
1.1e+08
1e+08
9e+07
8e+07
7e+07
6e+07
5e+07
4e+07
3e+07
2e+07
1e+07
5.00 10.00 15.00 20.00 25.00 30.00 35.00 40.00 45.00 50.00 55.00
Time-->


Abundance
TIC: T070404.D
7e+07
6.5e+07
6e+07
5.5e+07
5e+07
4.5e+07
4e+07
3.5e+07
3e+07
2.5e+07
2e+07
1.5e+07
1e+07
5000000
Chloroalkanes
5.00
10.00
15.00
20.00
25.00
30.00
35.00
40.00
Time-->

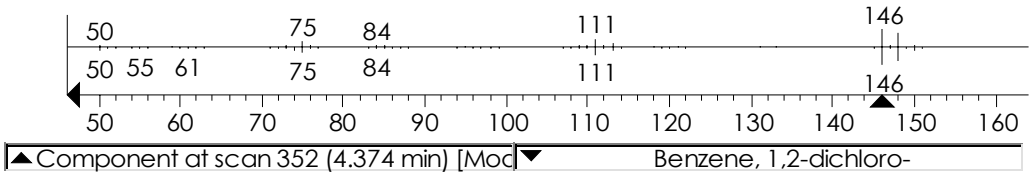

Abundance
TIC: T070401.D
8000000
7500000
7000000
6500000
6000000
5500000
5000000
4500000
4000000
3500000
3000000
2500000
2000000
1500000
1000000
500000
Triclosan
4.00
6.00
8.00
10.00
12.00
14.00
16.00
18.00
20.00
22.00
Time-->

Appendix III. Extracted mass spectra and comparison with NIST library

Examples of extracted mass spectra (upper part of figure) from different samples and 'head-to-tail' comparison with reference spectra from the NIST library (lower part of figure).

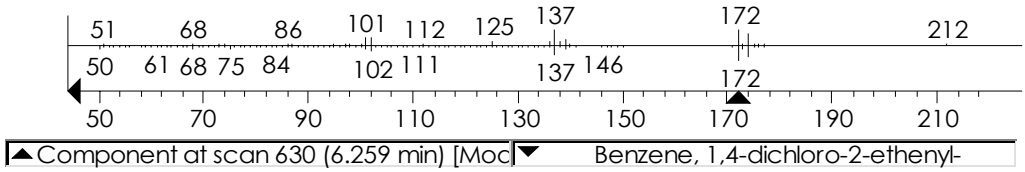
Dichloromethane

Benzene, 1,2-dichloro- RT=4.374



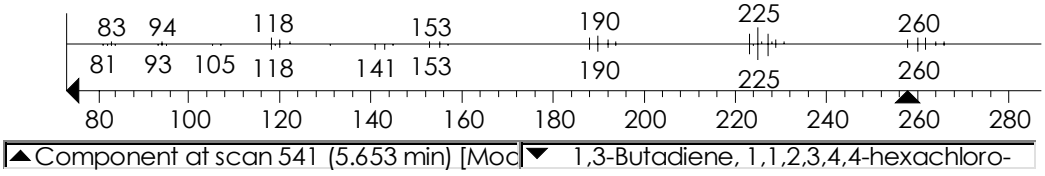
1,2-Dichloroethane

Benzene, 1,4-dichloro-2-ethenyl- RT= 6.259



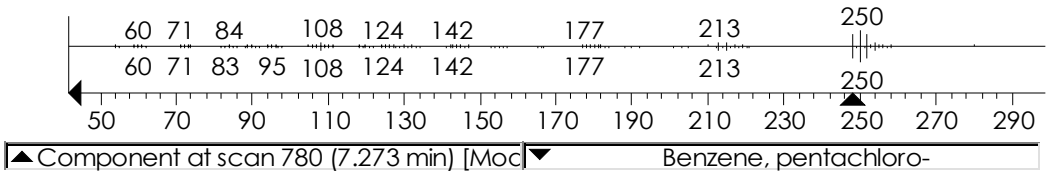
Perchloroethylene

1,3-Butadiene, 1,1,2,3,4,4-hexachloro- RT=5.653



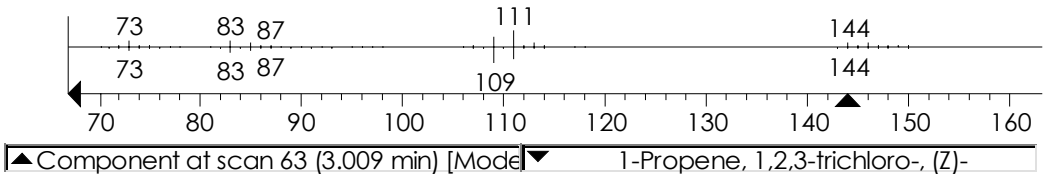
Trichloroethylene

Benzene, pentachloro- RT=7.273



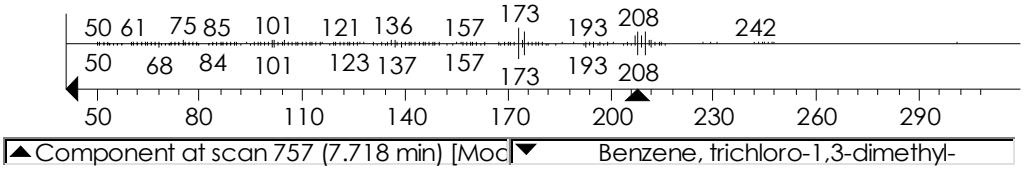
Allylchloride

1-propene, 1,2,3-trichloro- RT=3.009



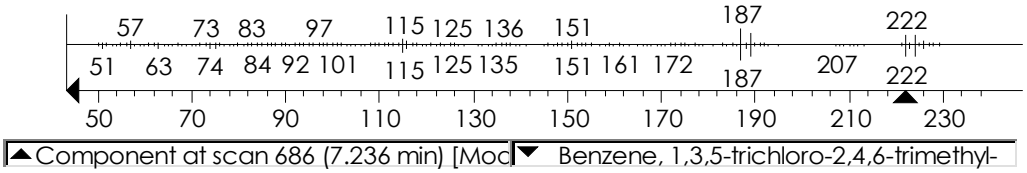
Hydrochloric acid

Benzene, trichloro-1,3-dimethyl- RT= 7.718



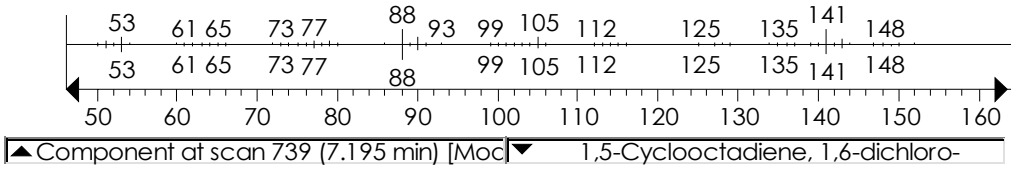
Monochloroacetic acid

Benzene, 1,3,5-trichloro-2,4,6-trimethyl- RT=7.236



Chloroprene rubber

1,5-Cyclooctadiene, 1,6-dichloro-RT=7.195



Appendix IV. Polychlorinated dioxins (PCDDs) and dibenozfurans (PCDFs)

Determind in selected products, responsive in the DR-CALUX® test.

Concentrations as reported as pg/g product for individual congeners and as the total 2,3,7,8-TCDD equivalent concentrations in pg WHO-TEQs/g product.

Congener	Concentration in pg/g product			WHO-TEF
	Poly-chloroprene	HCl	TRI	
2,3,7,8-T4CDD	<28	<0.16	<0.18	1
1,2,3,7,8-P5CDD	43	<0.19	0.47	1
1,2,3,4,7,8-H6CDD	<40	<0.35	<0.08	0.1
1,2,3,6,7,8-H6CDD	<40	<0.35	<0.08	0.1
1,2,3,7,8,9-H6CDD	<38	<0.38	<0.08	0.1
1,2,3,4,6,7,8-H7CDD	186	0.82	0.31	0.01
OCDD	713	2.97	0.91	0.0001
2,3,7,8-T4CDF	50	<0.12	<0.14	0.1
1,2,3,7,8-P5CDF	<25	0.22	<0.16	0.05
*1,2,3,4,8-P5CDF				
2,3,4,7,8-P5CDF	48	0.20	<0.11	0.5
1,2,3,4,7,8-H6CDF	39	0.53	0.72	0.1
*1,2,3,4,7,9-H6CDF				
1,2,3,6,7,8-H6CDF	34	0.42	0.18	0.1
1,2,3,7,8,9-H6CDF	39	0.54	0.60	0.1
2,3,4,6,7,8-H6CDF	42	0.63	0.18	0.1
1,2,3,4,6,7,8-H7CDF	70	0.54	1.25	0.01
1,2,3,4,7,8,9-H7CDF	<40	<0.5	0.66	0.01
OCDF	<59	0.89	0.53	0.0001
Sum PCDD	943	3.8	1.7	
Sum PCDF	321	4.0	4.1	
Sum PCDD/F	1263	7.8	5.8	
Sum WHO-TEQ (<DL as 0)	90	0.34	0.66	
Sum WHO-TEQ (<DL as DL)	132	0.8	0.9	

* Non-toxic isomer, usually present in low concentrations, that cannot be separated from related isomers with the current method.

Appendix V. Concentration units

Multipliers

The results have been expressed as far as possible on consistent concentrations units. As concentration levels reported varied over more than 9 orders of magnitude different units had to be applied for reasons of clarity of presentation. For readers not familiar with the different concentration units, a table with multipliers for conversion is indicated below.

Units	g	mg
mg	10^{-3}	1
µg	10^{-6}	10^{-3}
ng	10^{-9}	10^{-6}
pg	10^{-12}	10^{-9}

Concentration units of dioxins and DR-CALUX® response

Concentrations of dioxins, derived from chemical determinations with HRGCMS are usually expressed as toxic equivalent concentrations (TEQs) of the most toxic congener (2,3,7,8-TCDD) and, depending of the toxic equivalency factor (TEF) system used, indicated as I-TEQs (NATO/CCMS, 1988) or in more recent studies as WHO-TEQs (UNEP, 2001; Van den Berg et al., 1998). The differences between both systems are explained in the latter publications and De Koning (2004).

The response in the DR-CALUX® bioassays is expressed as a 2,3,7,8-TCDD equivalent concentration (see method section) and indicated in this report as DR-CALUX® based bio-TEQs, in order to avoid confusion. The response after acidic clean-up may be interpreted as being composed of contributions from dioxins, dioxin-like compounds (such as e.g. coplanar PCBs, PCNs), and non-halogenated transient inducers, that survive the acidic clean-up step (such as some PAHs), but that do no lead to the same range of typical effects as dioxins and dioxin-likes in *in-vivo* studies. Additional chemical confirmation of the contribution of these transient-inducers and their relative potency in the DR-CALUX® test is required, before the results can be attributed to dioxins or dioxin-likes. More background information on the significance and interpretation of the DR-CALUX® and other in-vitro assays is provided in the final summarizing report on the OVOC project.